BOX SEQ A

PATENT APPLICATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Docket No: 29473/35834

PATENT APPLICATION TRANSMITTAL UNDER 37 C.F.R. 1.53

Box Patent Application
Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

Transmitted herewith for filing is the patent application of

Inventors: Leopold FLOHÉ, Mahavir SINGH, Bernd HUTTER and Arend KOLK

Title: TEST KIT FOR TUBERCULOSIS DIAGNOSIS BY DETERMINING ALANINE DEHYDROGENASE

1. Type of Application

☒ This is a new application for a

☒ utility patent.

☐ design patent.

2. Application Papers Enclosed

1	Title Page
49	Pages of Specification (excluding Claims, Abstract, Drawings & Sequence Listing)
4	Pages of Claims
1	Page of Abstract
10	Sheets of Drawings (Figs. 2.1 to 3.19)
	☐ Formal
	☒ Informal
20	Pages of Sequence Listing

CERTIFICATION UNDER 37 CFR 1.10

I hereby certify that this Patent Application Transmittal and the documents referred to as enclosed therewith are being deposited with the United States Postal Service on **July 28, 1999**, in an envelope addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231 utilizing the "Express Mail Post Office to Addressee" service of the United States Postal Service under Mailing Label No. EM099539102US.

Richard Zimmermann

3. **Declaration or Oath**

☐ Enclosed

☐ Executed by (check all applicable boxes)

☐ Inventor(s)

☐ Legal representative of inventor(s)
(37 CFR 1.42 or 1.43)

☐ Joint inventor or person showing a proprietary interest on behalf of inventor who refused to sign or cannot be reached

☐ The petition required by 37 CFR 1.47 and the statement required by 37 CFR 1.47 are enclosed. See Item 5D below for fee.

☒ Not enclosed - the undersigned attorney or agent is authorized to file this application on behalf of the applicants. An executed declaration will follow.

4. **Additional Papers Enclosed**

☒ Preliminary Amendment

☐ Information Disclosure Statement

☐ Declaration of Biological Deposit

☒ Computer readable copy of sequence listing containing nucleotide and/or amino acid sequence

☐ Microfiche computer program

☐ Verified statement(s) claiming small entity status under 37 CFR 1.9 and 1.27

☐ Associate Power of Attorney

☐ Verified translation of a non-English patent application

☐ An assignment of the invention

☒ Return receipt postcard

☐ Other

5. **Priority Applications Under 35 USC 119**

Certified copies of applications from which priority under 35 USC 119 is claimed are listed below and

☐ are attached.

☒ will follow.

COUNTRY	APPLICATION NO.	FILED
EPO	97 101 338.8	29 January 1997

6. **Filing Fee Calculation (37 CFR 1.16)**

A. ☒ **Utility Application**

CLAIMS AS FILED - INCLUDING PRELIMINARY AMENDMENT (IF ANY)						
			SMALL ENTITY		OTHER THAN A SMALL ENTITY	
	NO. FILED	NO. EXTRA	RATE	FEE	RATE	FEE
BASIC FEE				$380.00		$760.00
TOTAL	18 -20	= 0	X 9 =		X 18 =	
INDEP.	1 - 3	= 0	X 39 =		X 78 =	
☐ First Presentation of Multiple Dependent Claim			+ 130 =		+ 260 =	
Filing Fee:					**OR**	$760.00

B. ☐ **Design Application ($155.00/$310.00)** Filing Fee: $ _____

C. ☐ **Plant Application ($240.00/$480.00)** Filing Fee: $ _____

D. **Other Fees**

☐ Recording Assignment [Fee -- **$40.00** per assignment] $ _____

☐ Petition fee for filing by other than all the inventors or person on behalf of the inventor where inventor refused to sign or cannot be reached [Fee -- **$130.00**] $ _____

☐ Other $ _____

Total Fees Enclosed $760.00

7. **Method of Payment of Fees**

☒ Enclosed check in the amount of: $760.00

☐ Charge Deposit Account No. 13-2855 in the amount of: $_____
A copy of this Transmittal is enclosed.

☐ Not enclosed

8. **Deposit Account and Refund Authorization**

The Commissioner is hereby authorized to charge any deficiency in the amount enclosed or any additional fees which may be required during the pendency of this application under 37 CFR 1.16 or 37 CFR 1.17 or under other applicable rules (except payment of issue fees), to Deposit Account No. 13-2855. A copy of this Transmittal is enclosed.

Please refund any overpayment to Marshall, O'Toole, Gerstein, Murray & Borun at the address below.

Please direct all future communications to James P. Zeller, at the address below.

Respectfully submitted,

MARSHALL, O'TOOLE, GERSTEIN,
MURRAY & BORUN
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By: _____
James P. Zeller
Reg. No: 28,491

July 28, 1999

PATENT

IN THE UNITED STATES PATENT
AND TRADEMARK OFFICE

Applicant: Flohé et al. Serial No.: To be assigned Filed: Herewith (July 28, 1999) For: TEST KIT FOR TUBERCULOSIS DIAGNOSIS BY DETERMINING ALANINE DEHYDROGENASE Continuation of PCT/EP98/00483 filed January 29, 1998 Group Art Unit: To be assigned Examiner: To be assigned	"EXPRESS MAIL" mailing label No. . Date of Deposit: July 28, 1999 I hereby certify that this paper (or fee) is being deposited with the United States Postal Service "EXPRESS MAIL POST OFFICE TO ADDRESSEE" service under 37 CFR §1.10 on the date indicated above and is addressed to: Assistant Commissioner for Patents, Washington, D.C. 20231 Richard Zimmermann

PRELIMINARY AMENDMENT

Assistant Commissioner for Patents
Washington, DC 20231

Sir:

Please amend this application as follows.

IN THE SPECIFICATION:

Page 1, line 1 please delete "**Test kit for tuberculosis diagnosis etc.**" and substitute --Test Kit for Tuberculosis Diagnosis by Determining Alanine Dehydrogenase--.

Page 1, immediately following the title please insert the following:

--This is a continuation of International Application No. PCT/EP98/00483 filed January 29, 1998 (the entire disclosure of which is incorporated herein by reference.--

IN THE ABSTRACT:

Please add an abstract to the disclosure on a separate sheet (attached hereto).

IN THE CLAIMS:

Please amend claims 2-16 as follows:

2. (Amended) A method for the diagnosis of tuberculosis and other mycobacterial infections of humans and animals, [**characterised** in that] comprising the step of measuring the activity of alanine dehydrogenase (E.C. 1.4.1.1.) [is measured] with an enzymatic test kit according to claim 1.

3. (Amended) A method according to claim 2, [**characterised** in that] comprising the steps of

(i) isolating possible tuberculosis pathogens, [such as *M. tuberculosis*, are isolated,]

(ii) making a crude cell extract [is made],

(iii) incubating the extract [is incubated] in solution, and

(iv) measuring the absorption [is measured].

4. (Amended) A method according to claim 2 [and/or 3, **characterised** in that] comprising the steps of subjecting clinical samples[, such as body fluids, are subjected] directly to tuberculosis diagnosis and measuring the alanine dehydrogenase activity [is measured].

5. (Amended) A method according to claim 2, [**characterised** in that] comprising the step of differentiating at least one of cells, strains [and/or] and species of disease-causing organisms (mycobacteria) [are differentiated] from non-virulent cells and strains.

6. (Amended) A method according to claim 5, [**characterised** in that] comprising the steps of identifying and differentiating at least one of cells, strains [and/or] and species of disease-causing organisms of the *M. tuberculosis* complex [are identical and differentiated].

7. (Amended) A method according to [any one of the preceding claims, **characterised** in that the method is carried] claim 2, wherein said steps are carried out in the presence of substances that inhibit at least one of tuberculosis and other mycobacterial infections of humans and animals and [those] optionally recovering said inhibiting substances [are optionally recovered].

8. (Amended) A method according to [any one of the preceding claims, **characterised** in that it is] claim 2, wherein said steps are carried out

(i) to control epidemics and/or

(ii) after vaccinations (vaccination follow-up) in humans [and] or animals.

9. (Amended) A DNA sequence selected from the group consisting of the following [group or] partial sequences and other partial sequences of the alanine dehydrogenase gene of *M. tuberculosis* (Fig. 2.5):

Name	Sequence	Orientation
AlaDH-F1	5'-ATGCGCGTCGGTATTCCG-3'	forward
AlaDH-F1+	5'-GCGCGTCGGTATTCCGACCG-3'	forward
AlaDH-F2	5'-GAGACCAAAACAACGAA-3'	forward
AlaDH-F4	5'-GAATTCCCATCAGCAATCTTGCAGA-3'	forward
AlaDH-F5	5'-GCCCCGATGAGCGAAGTC-3'	forward
AlaDH-F6	5'-GGGGCCGTCCTGGTGCC-3'	forward
AlaDH-F7	5'-GACGTCGACCTACGCGCTGAC-3'	forward
AlaDH-R1	5'-CTCGGTGAACGGCACCCC-3'	reverse
AlaDH-R2	5'-GGCCAGCACGCTGGCGGG-3'	reverse
AlaDH-R3	5'-CACCCGTTCGGACAGTAA-3'	reverse
AlaDH-R4	5'-CGCGGCCGACATCATCGC-3'	reverse
AlaDH-R5	5'-GGCCGACATCATCGCTTCCC-3'	reverse
AlaDH-R6	5'-CGAGACTAATTTGGGTGCCTTGGC-3'	reverse
AlaDH-R7	5'-ATTTGGGTGCCTTGGC-3'	reverse
AlaDH-RM	5'-GGCGGCGAGTCGACCGGC-3'	reverse

[and], partial sequences thereof and sequences that are [hybridisable] hybridizable therewith [preferably at a temperature of at least 20°C and especially at a concentration of 1M NaCl and a temperature of at least 25°C], for the diagnosis of tuberculosis and other mycobacterial infections in humans [and] or animals.

10. (Amended) [The use of a DNA sequence according to claim 9] A method for the diagnosis of tuberculosis and other mycobacterial infections in humans and animals comprising the step of using said DNA sequence of claim 9 in said diagnosis.

11. (Amended) A method according to claim 10, [**characterised** in that a] comprising the step of using said DNA sequence [according to claim 9 is used] for at least one of

(i) [for hybridisation] hybridization,

(ii) [for] culture confirmation of isolated strains [and/or] and,

(iii) [for] chromosomal fingerprinting, and

comprising the step of determining and differentiating at least one of cells, strains [and/or] and types of mycobacteria [are determined and differentiated] and/or [are used for the diagnosis of] diagnosing mycobacterial infections.

12. (Amended) A method according to claim 10 [or 11, **characterised** in that] comprising the step of differentiating at least one of cells, strains [and/or] and species of virulent mycobacteria [are differentiated] from non-virulent cells, strains and/or species.

13. (Amended) A method according to claim 10, [**characterised** in that cells, strains and/or species of the *M. tuberculosis* complex and other mycobacteria] comprising the steps of

(i) [are isolated] isolating cells, strains and/or species of at least one of the *M. tuberculosis* complex and other mycobacteria,

(ii) recovering crude or purified genomic DNA or RNA [is recovered], and,

(iii) identifying a fragment that is identical or virtually identical to the sequence of the alanine dehydrogenase gene of *M. tuberculosis* (Fig. 2.3) [is identified, preferably by amplification using a DNA sequence according to claim 9 as a primer sequence, after which digestion is carried out with a restriction enzyme, especially Bg1II, and gel electrophoresis of the digested amplified DNA is carried out and/or the DNA sequence of the amplified DNA is determined].

14. (Amended) A method according to claim 2 [and/or 10, **characterised** in that] comprising the step of

diagnosing a clinical sample [is used directly and diagnosed] for tuberculosis in humans [and] or animals.

15. (Amended) A method according to claim 2 [and/or 10, **characterised** in that the method is] carried out in the presence of substances that inhibit tuberculosis or mycobacterial infections of humans [and] or animals and comprising the step of determining and recovering or making inhibiting substances [determined are recovered or made].

16. (Amended) A method according to claim 10[, **characterised** in that it is] used in at least one of

(i) [in] antimycobacterial chemotherapy,

(ii) [in] the control of epidemics [and/or] and

(iii) after vaccinations (vaccination follow-up) in humans [and] or animals.

Please add new claims 17 and 18 as follows:

17. A method according to claim 3 wherein the pathogen is *M. tuberculosis*.

18. A method according to claim 4 wherein the clinical sample is a body fluid.

REMARKS

By the foregoing, page 1 of the specification has been amended to provided the full title and a cross-reference to the parent international application. An abstract has been added, and the claims have been amended for conformity with U.S. practice and to eliminate multiple dependencies. New claims 17 and 18, based on original claims 3 and 4, respectively, have been added.

The filing fee for this application has been calculated based on the claims as amended above.

Respectfully submitted,

MARSHALL, O'TOOLE, GERSTEIN,
MURRAY & BORUN

July 28, 1999 By _____
James P. Zeller
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JOINT INVENTORS

"EXPRESS MAIL" mailing label No. EM099539102US.
Date of Deposit: July 28, 1999
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Richard Zimmermann

APPLICATION FOR UNITED STATES LETTERS PATENT

SPECIFICATION

TO ALL WHOM IT MAY CONCERN:

Be it known that we, Leopold FLOHÉ, a citizen of Germany, residing at Vogelsang 5, D-38304 Wolfenbüttel, Germany; Mahavir SINGH, a citizen of Germany, residing at Mascheroder Weg 1, D-38124 Braunschweig, Germany; Bernd HUTTER, a citizen of Germany, residing at Mascheroder Weg 1, D-38124 Braunschweig, Germany; and Arend KOLK, a citizen of Germany, residing at Mascheroder Weg 1, D-38124 Braunschweig, Germany have invented a new and useful TEST KIT FOR TUBERCULOSIS DIAGNOSIS BY DETERMINING ALANINE DEHYDROGENASE, of which the following is a specification.

Test kit for tuberculosis diagnosis etc.

Isolated lambda gt11 clones containing the complete AlaDH coding DNA of *M. tuberculosis* or parts thereof are known from Anderson *et al.* (1992). The isolated mycobacterial AlaDH insert from lambda AA67 was used as the hybridisation probe in that work.

1 Problem and Invention

The 40 kD antigen with which this work is concerned is in many respects an interesting subject for detailed studies.

The antigen had already been cloned into an expression vector for *Escherichia coli* (Konrad & Singh, unpublished). The expression and purification of the recombinant protein was therefore to be optimised. Using a homogeneous protein fraction, the crucial biochemical parameters of the enzyme were then to be determined. Previous experience has shown that it is possible to infer the physiological function of an enzyme from such data. The question that this posed was whether the hypothetical function of the enzyme in cell wall biosynthesis could be confirmed or disproved. If disproved, other possible functions were to be elicited.

In addition, the biochemistry may provide starting points for specific influencing of the enzyme *in vivo*. In that context, the physiological function is once again the key point for all efforts towards that end. If the antigen were to play an essential role for the bacterium, then attempts aimed specifically at switching off the gene or the protein might provide possibilities for preventing the growth of the tuberculosis pathogen at a defined point. The protein would then be an ideal drug target. If, in addition, as postulated (Delforge *et*

al., 1993), the 40 kD antigen were to represent a virulence factor, influence might be brought to bear on the natural virulence of the bacterium by such endeavours. That aspect also was to be verified, therefore, by various tests.

The ability to discriminate the strains *M. tuberculosis* and *M. bovis* BCG by means of the mAb HBT-10 makes it possible to develop methods of distinguishing an infection from a vaccination. That is not possible with the conventional screening methods, the PPD and the Mantoux test (Bass Jr. *et al.*, 1990; Huebner *et al.*, 1993). By analysis of the distribution of the gene or the gene product the foundation was to be laid for the development of an economical method for such a test. In addition, whether the presence of a functional enzyme correlates with any other parameters was to be investigated. Particular importance was attached to correlations between taxonomy and virulence. Certain natural modes of life or the entry into certain growth phases might also be related to alanine dehydrogenase. Fundamental answers were to be sought to those questions.

The invention relates to an enzymatic test kit for the diagnosis of tuberculosis and other mycobacterial infections in humans and animals by determination of the activity of alanine dehydrogenase (E.C. 1.4.1.1), comprising L-alanine, nicotinamide adenine dinucleotide (oxidised form; NAD^+), phenazine methosulphate (PMS) and nitroblue tetrazolium chloride (NBT).

The invention further relates to a method of diagnosing tuberculosis and other mycobacterial infections of humans and animals, ***characterised*** in that the activity of alanine dehydrogenase (E.C. 1.4.1.1.) is measured with an enzymatic test kit according to claim 1.

The method according to the invention may be ***characterised*** in that

(i) possible tuberculosis pathogens, such as *M. tuberculosis*, are isolated,
(ii) a crude cell extract is made,
(iii) the extract is incubated in solution and
(iv) the absorption is measured.

The method according to the invention may further be ***characterised*** in that clinical samples, such as body fluids, are subjected directly to tuberculosis diagnosis and the alanine dehydrogenase activity is measured.

The method according to the invention may further be ***characterised*** in that cells, strains and/or species of disease-causing organisms (mycobacteria) are differentiated from non-virulent cells and strains.

The method according to the invention may further be ***characterised*** in that cells, strains and/or species of disease-causing organisms of the *M. tuberculosis* complex are identified and differentiated.

The method according to the invention may further be ***characterised*** in that the method is carried out in the presence of substances that inhibit tuberculosis and other mycobacterial infections of humans and animals and those inhibiting substances are optionally recovered.

The method according to the invention may further be ***characterised*** in that it is carried out

(i) to control epidemics and/or
(ii) after vaccinations (vaccination follow-up) in humans and animals.

The invention further relates to a DNA sequence selected from the following group or other partial sequences of the alanine dehydrogenase gene of *M. tuberculosis* (Fig. 2.5):

Name	Sequence	Orientation
AlaDH-F1	5'-ATGCGCGTCGGTATTCCG-3'	forward
AlaDH-F1+	5'-GCGCGTCGGTATTCCGACCG-3'	forward
AlaDH-F2	5'-GAGACCAAAACAACGAA-3'	forward
AlaDH-F4	5'-GAATTCCCATCAGCAATCTTGCAGA-3'	forward
AlaDH-F5	5'-GCCCCGATGAGCGAAGTC-3'	forward
AlaDH-F6	5'-GGGGCCGTCCTGGTGCC-3'	forward
AlaDH-F7	5'-GACGTCGACCTACGCGCTGAC-3'	forward
AlaDH-R1	5'-CTCGGTGAACGGCACCCC-3'	reverse
AlaDH-R2	5'-GGCCAGCACGCTGGCGGG-3'	reverse
AlaDH-R3	5'-CACCCGTTCGGACAGTAA-3'	reverse
AlaDH-R4	5'-CGCGGCCGACATCATCGC-3'	reverse
AlaDH-R5	5'-GGCCGACATCATCGCTTCCC-3'	reverse
AlaDH-R6	5'-CGAGACTAATTTGGGTGCCTTGGC-3'	reverse
AlaDH-R7	5'-ATTTGGGTGCCTTGGC-3'	reverse
AlaDH-RM	5'-GGCGGCGAGTCGACCGGC-3'	reverse

and partial sequences thereof and sequences that are hybridisable therewith preferably at a temperature of at least 20°C and especially at a concentration of 1M NaCl and a temperature of at least 25°C, for the diagnosis of tuberculosis and other mycobacterial infections in humans and animals.

The use according to the invention of a DNA sequence may be envisaged for the diagnosis of tuberculosis and other mycobacterial infections in humans and animals.

The invention further relates to a method that is ***characterised*** in that a DNA sequence according to the invention is used

(i) for hybridisation,
(ii) for culture confirmation of isolated strains and/or
(iii) for chromosomal fingerprinting, and cells, strains and/or types of mycobacteria are determined and differentiated and/or are used for the diagnosis of mycobacterial infections.

The method according to the invention may be ***characterised*** in that cells, strains and/or species of virulent mycobacteria are differentiated from non-virulent cells, strains and/or species.

The method according to the invention may further be ***characterised*** in that cells, strains and/or species of the *M. tuberculosis* complex and other mycobacteria
(i) are isolated,
(ii) crude or purified genomic DNA or RNA is recovered,
(iii) a fragment that is identical or virtually identical to the sequence of the alanine dehydrogenase gene of *M. tuberculosis* (Fig. 2.3) is identified, preferably by amplification using a DNA sequence according to the invention as a primer sequence, after which digestion is carried out with a restriction enzyme, especially Bg1II, and gel electrophoresis of the digested amplified DNA is carried out and/or the DNA sequence of the amplified DNA is determined.

The method according to the invention may further be ***characterised*** in that a clinical sample is used directly and diagnosed for tuberculosis in humans and animals.

The method according to the invention may further be ***characterised*** in that the method is carried out in the presence of substances that inhibit tuberculosis or mycobacterial infections of humans and animals and inhibiting substances deter-

mined are recovered or made.

The method according to the invention may further be ***characterised*** in that it is used

(i) in antimycobacterial chemotherapy,

(ii) in the control of epidemics and/or

(iii) after vaccinations (vaccination follow-up) in humans and animals.

2 Materials and Methods

2.1 Living Material

2.1.1 Bacteria

2.1.1.1 *E. coli* strains

The strain *Escherichia coli* was used to optimise the expression of the recombinant 40 kD antigen (**Tab. 2.1**). In addition, mycobacterial antigens already cloned therein were over-produced **(Tab. 2.2)**.

Tab. 2.1: *Expression strains used and their relevant properties*

strain	genotype and relevant phenotype	origin / reference
E.coli CAG 629	*lac*(am) *pho*(am) *trp*(am) *sup*C^{ts} *rps*L *mal*(am) *lon* *htp*RI65-Tn10(Tet^R)	C.Gross
E. coli DH5α	*sup*E44 Δ*lac*UI69(ϕ80 *lac*Z ΔM15) *hsd*RI7 *rec*A1 *end*A1 *gyr*A96 *thi*-1 *rel*A1	Hanahan(1983)
E. coli TG2	*sup*E *hsd*Δ5 *thi*Δ(*lac-pro*AB) Δ(*srl-rec*A)306::Tn10(Tet^R) F'(*tra*D36 *pro*A$^+$ *lacI*^{q} *lac*ZM 15)	Sambrook *et al.*(1989)
E.coli SURE	*hsd*R *mcr*A *mcr*B *mvr* *end*A *sup*E44 *thi*-1 λ- *gyr*A96 *rel*A1 *lac* *rec*B *rec*J *sbc*C *umu*C *uvr*C (F' *pro*AB *lacI*^{q}Z ΔM15 Tn10(Tet^R))	Stratagene
E. coli BL 321	*rnc*105 *nad*B$^+$ *pur*I$^+$	Studier (1975)
E. coli N 4830	*su*^{o} *his* *ilv* *gal*KΔ8 ΔchlD-*pgl* (λ ΔBam N$^+$ cI$_{ts857}$ ΔHI)	Gottesman *et al.* (1980)
E. coli 538	genotype unknown	Bayer AG

Tab. 2.2 (1/2): *Producers of mycobacterial antigens and characteristics thereof*

The antigen produced by the respective strain is indicated. The last two columns give the growing conditions.

Strain	origin / reference(s)	product	antibiotics	induction
E. coli BL21 (pKAM1301)	J. van Embden	GST-36 kD antigen, *M. leprae*	Ap	IPTG
E. coli BL21/plys 5 (pKAM3601)	J. van Embden	70 kD antigen, *M. leprae*	Ap + Cm	IPTG
E. coli CAG629 (pMS9-2)	Singh *et al.* (1992)	38 kD antigen, *M. tuberculosis*	Ap	heat
E. coli CAG629 (pMS14-1)	Cherayil & Young (1988) Dale & Patki (1990) Singh *et al.* (unpublished)	28 kD antigen, *M. leprae*	Ap	heat
E. coli M15 (pHISK16 + pREP4)	Verbon *et al.* (1992) Vordermeier *et al.* (1993)	16 kD antigen, *M. tuberculosis*	Ap	IPTG
E. coli M1697	V. Mehra	His-30 kD antigen, *M. tuberculosis*	Ap + Km	IPTG
E. coli M1698	V. Mehra	His-30 kD antigen, *M. leprae*	Ap + Km	IPTG
E. coli POP (pKAM2101)	J. van Embden	70 kD antigen, *M. tuberculosis*	Ap	heat
E. coli POP (pRIB1300)	Thole *et al.* (1987) van Eden *et al.* (1988)	65 kD antigen, *M. bovis* BCG	Ap	heat
E. coli POP (pZW1003)	Mehra *et al.* (1986) van der Zee *et al.* (unpublished)	65 kD antigen, *M. leprae*	Ap	heat
E. coli TB1 (pKAM1101)	di Guan *et al.* (1987) Maina *et al.* (1988) Thole *et al.* (1990)	MBP-38 kD antigen, *M. leprae*	Ap	heat

Tab. 2.2 (2/2): *Producers of mycobacterial antigens and characteristics thereof*

The antigen produced by the respective strain is indicated. The last two columns give the growing conditions.

Strain	origin / reference(s)	product	antibiotics	induction
E. coli TB1 (pKAM1401)	J. van Embden	MBP-2nd 65 kD antigen, *M. leprae*	Ap	heat
E. coli TB21-8/2	Khanolar-Young *et al.* (1992) Mehra *et al.* (1992)	MBP-10 kD antigen, *M. tuberculosis*	Ap	IPTG
E. coli TG2 - 50/55 Sal large	C. Espitia; M. Singh	50/55 kD, large frag., *M. tuberculosis*	Ap	IPTG

2.1.1.2 Mycobacterial strains

Tab. 2.3 (1/3): *Mycobacteria used and the origin thereof*

strain	abbreviation	exact name, origin
M. africanum 1	Afr1	*M. africanum* No. 5544, RIV
M. asiaticum 1	Asi1	*M. asiaticum* 3250, Portaals
M. avium 1	Avi1	*M. avium* Myc 3875, Serotype 2, RIV
M. bovis 3	Bov3	*M. bovis* No. 8316, RIV
M. bovis BCG 2	BCG2	*M. bovis* Copenhagen, Seruminstitut Copenhagen
M. bovis BCG 4	BCG4	*M. bovis* BCG P_3, RIV
M. chelonae 7	Che7	*M. chelonei* 1490, P. Dirven
M. flavescens 1	Fla1	*M. flavescens* ATCC 14474, RIV
M. fortuitum 11	For11	*M. fortuitum* ATCC 6841, RIV
M. gastri 1	Gas1	*M. gastri* ATCC 25220, RIV
M. gordonae 3	Gor3	*M. gordonae* 8690, Portaals

Tab. 2.3 (2/3): Mycobacteria used and the origin thereof

strain	abbreviation	exact name, origin
M. intracellulare 1	Int1	*M. intracellulare* 6997, ATCC 15985, Portaals
M. intracellulare 5	Int5	*M. intracellulare* IWG MT3, RIV
M. kansasii 1	Kan1	*M. kansasii* Myc 1012, RIV
M. lufu 1	Luf1	*M. lufu* 219, RIV
M. marinum 3	Mar3	*M. marinum* L66, Portaals
M. microti 1	Mic1	*M. microti* No. 1278, Portaals
M. nonchromogenium 1	Non1	*M. nonchromogenium* ATCC 25145, RIV
M. parafortuitum 1	Paf1	*M. parafortuitum* No. 6999, Portaals
M. peregrinum 1	Per1	*M. peregrinum*, Patient Bakker, TB6849, Antonie Ziekenhius
M. phlei 1	Phl1	*M. phlei* 258 (Ph), Portaals
M. phlei 4	Phl4	*M. phlei* Weybridge R82, Tony Eger
M. scrofulaceum 1	Scr1	*M. scrofulaceum* Myc 3442, RIV
M. scrofulaceum 8	Scr8	*M. scrofulaceum* Myc 6672, RIV
M. simiae 1	Sim1	*M. simiae* 784, Tony Eger
M. smegmatis 1	Sme1	*M. smegmatis* ATCC 14460, RIV
M. smegmatis 3	Sme3	*M. smegmatis* 8070, Portaals
M. terrae 2	Ter2	*M. terrae*, RIV
M. thermoresistibile 1	The1	*M. thermoresistibile* No. 7001, Portaals
M. triviale 1	Tri1	*M. triviale* 8067, Portaals
M. tuberculosis $H37R_v$	$H37R_v$	*M. tuberculosis* $H37R_v$, RIV
M. tuberculosis $H37R_a$	$H37R_a$	*M. tuberculosis* $H37R_a$, No. 19629, RIV
M. tuberculosis 1	Tub1	*M. tuberculosis* 4514, RIV
M. tuberculosis 49	Tub49	*M. tuberculosis* C_3, Sang-Hae Cho, South Korea
M. tuberculosis 60	Tub60	*M. tuberculosis* S_2, Sang-Hae Cho, South Korea

Tab. 2.3 (3/3): Mycobacteria used and the origin thereof

strain	abbreviation	exact name, origin
M. tuberculosis 118	Tub118	*M. tuberculosis* Myc 16293, Hannoufi
M. tuberculosis 130	Tub130	*M. tuberculosis*, Patient yy, barcode 3.1265, Dr. Bijlmer, The Hague
M. tuberculosis 132	Tub132	*M. tuberculosis* Myc 16770, RIV
M. tuberculosis 145	Tub145	*M. tuberculosis* 416138N, Patient N.Wielaart, Reg. No. 7.796.267, WKZ, Utrecht
M. tuberculosis 146	Tub146	*M. tuberculosis*, Abdi Hussein
M. tuberculosis 163	Tub163	*M. tuberculosis* 925, patient isolate No. 32, INH>1, Str^R, Rif^S, Eth^S
M. ulcerus 1	Ulc1	*M. ulcerus* 932, Portaals
M. vaccae 3	Vac3	*M. vaccae* ATCC 25950, RIV
M. xenopi 7	Xen7	*M. xenopi* code 132, Patient Alois Necas, H. Kristanpul, Prague

2.1.1.3 Other strains of bacteria

Tab. 2.4: Other strains of bacteria used

strain	origin
Listeria monocytogenes EGB	Andreas Lignau
Listeria innocua	Andreas Lignau
Nocardia asteroides 702774	Juul Bruins
Rhodococcus equi No. 10P388	VMDC, Utrecht

2.1.2 Cell culture

The mouse macrophage cell line J774 was used. That cell line was originally established from a tumour of a female BALB/c mouse (Ralph & Nakoinz, 1975). J774 is used for phagocytosis assays, for the production of IL-1 and for a wide range of

biochemical investigations. It has receptors for immunoglobulins and complement. J774 furthermore produces lysozyme in large quantities and secretes IL-1 constitutively (Ralph & Nakoinz, 1976; Snyderman *et al.*, 1977). Bacteria are taken up by phagocytosis. Direct cytolysis of foreign organisms is relatively rare.

2.2 Nucleic acids

2.2.1 Plasmids

Plasmid pJLA604Not and its relevant functional segments

This 4.9 kb plasmid, a derivative of pJLA 604 (Schauder *et al.*, 1987), was used as an expression vector (**Fig. 2.1**). The plasmid pJLA604Not (Konrad & Singh, unpublished) differs from pJLA604 in that the *NdeI* cleavage site has been removed and, in its place, a NotI cleavage site has been incorporated. The reading frame of the translation begins with the ATG codon of the SphI cleavage site. Transcription starts at the lambda promoters P_R and P_L, but is effectively repressed at temperatures of 28-30°C by the cI_{ts857}-gene product. Induction is achieved by increasing the temperature to 42°C. At that temperature, the temperature-sensitive lambda repressor becomes inactive and is no longer able to repress the transcription. Transcription ends at the fd terminator. In addition, the vector possesses the *atpE* translation initiation region (TIR) of *E. coli*. This segment is very useful for initiating translation since it has secondary structures that cause only little interference and consequently guarantees a high expression rate (McCarthy *et al.*, 1986). As a selection marker, the plasmid has at its disposal the β-lactamase gene that codes for ampicillin resistance.

As a negative control plasmid, pJLA603 also was used, which is identical to pJLA604 apart from a few bases in the cloning site.

Plasmid pMSKS12 and its relevant functional segments

This is a derivative of the plasmid pJLA604Not, in which the 40 kD antigen of *Mycobacterium tuberculosis* has been cloned between the *SphI* and the NotI cleavage sites (**Fig. 2.2;** Konrad & Singh, unpublished).

2.2.2 Oligonucleotides

All of the oligonucleotides (**Tab. 2.5)** were made by Frau Astrid Hans (GBF, Braunschweig) on a 394 DNA/RNA Synthesizer (Applied Biosystems). The oligonucleotides were purified with an Oligonucleotide Purification Cartridge (Applied Biosystems).

Tab. 2.5 (1/2): Oligonucleotides used

name	sequence	orientation
AlaDH-F1	5'-ATGCGCGTCGGTATTCCG-3'	forward
AlaDH-F1+	5'-GCGCGTCGGTATTCCGACCG-3'	forward
AlaDH-F2	5'-GAGACCAAAAACAACGAA-3'	forward
AlaDH-F4	5'-GAATTCCCATCAGCAATCTTGCAGA-3'	forward
AlaDH-F5	5'-GCCCCGATGAGCGAAGTC-3'	forward
AlaDH-F6	5'-GGGGCCGTCCTGGTGCC-3'	forward
AlaDH-F7	5'-GACGTCGACCTACGCGCTGAC-3'	forward
AlaDH-R1	5'-CTCGGTGAACGGCACCCC-3'	reverse
AlaDH-R2	5'-GGCCAGCACGCTGGCGGG-3'	reverse
AlaDH-R3	5'-CACCCGTTCGGACAGTAA-3'	reverse
AlaDH-R4	5'-CGCGGCCGACATCATCGC-3'	reverse
AlaDH-R5	5'-GGCCGACATCATCGCTTCCC-3'	reverse
AlaDH-R6	5'-CGAGACTAATTTGGGTGCCTTGGC-3'	reverse
AlaDH-R7	5'-ATTTGGGTGCCTTGGC-3'	reverse
AlaDH-RM	5'-GGCGGCGAGTCGACCGGC-3'	reverse

The location of the oligos on the *AlaDH* gene is shown schematically in **Fig. 2.3.** *(The oligos used and their position on the AlaDH gene)*

2.3 Formulations

All of the solutions described in this section were prepared very largely in accordance with Sambrook *et al.* (1989).

2.3.1 Nutrient media

LB

10 g of Bacto Tryptone (Difco), 5 g of Bacto yeast extract (Difco), 10 g of NaCl *ad* 1000 ml of H_2O, pH 7.0, autoclaving

IB

12 g of Bacto Tryptone (Difco), 24 g of Bacto yeast extract (Difco), 4 ml of glycerol (87 %), 2.31 g of KH_2PO_4, 12.54 g of K_2HPO_4 *ad* 1000 ml of H_2O, the phosphate solutions are separated from the other components, autoclaved and subsequently admixed

SOC

2 % Bacto Tryptone (Difco), 0.5 % Bacto yeast extract (Difco), 10 mM NaCl, 2.4 mM KCl, 10 mM $MgCl_2$, 10 mM $MgSO_4$, 20 mM glucose *ad* 1000 ml of H_2O, pH 7.0, the glucose is separated from the other components, autoclaved and subsequently added

LÖWENSTEIN

Ready-for-use Coletsos Ossein slant agar tubes (Sanofi Diagnostics Pasteur) were used.

SOLID MEDIA

To produce plates (90 mm, Greiner) of the nutrient media described above, 1.5 % agar was admixed with the relevant formulation.

ANTIBIOTICS

Antibiotics were added from stock solutions to the liquid media shortly before use. When producing solid media, the addition was delayed until the solution was hand-hot after autoclaving. The antibiotics listed in **Tab. 2.6** were used.

Tab. 2.6: Antibiotics used and concentrations employed

antibiotic	final concentration	dissolved in
ampicillin	100 µg/ml	water
chloramphenicol	20 µg/ml	ethanol
gentamicin	100 µg/ml	ready-for-use (Sigma)
kanamycin	30 µg/ml	water

2.3.2 Buffer solutions

L-BUFFER: 50 mM Tris base, 10 mM EDTA, pH 6.8, autoclaving

TE: 10 mM Tris base, 1 mM EDTA, pH 7.4, autoclaving

TAE: 40 mM Tris acetate, 1 mM EDTA, pH 8.0, autoclaving

TBE: 89 mM Tris base, 89 mM boric acid, 2 mM EDTA, pH 8.0

TBS: 50 mM Tris base, 137 mM NaCl, 3 mM KCl, pH 7.4, autoclaving

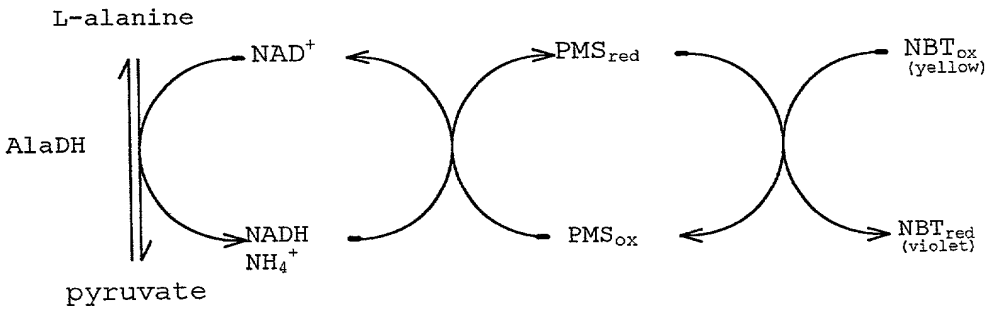
TBS-TWEEN: TBS + 0.05 % Tween-20

PBS: 137 mM NaCl, 3 mM KCl, 8 mM Na_2HPO_4, 2 mM KH_2PO_4, pH 7.0, autoclaving

2.4 Alanine Dehydrogenase Assays

2.4.1 Qualitative Assay

Qualitative detection of AlaDH is based on a number of redox reactions in accordance with the following reaction scheme (Inagaki *et al.*, 1986; Andersen *et al.*, 1992):



Principle of the alanine dehydrogenase assay

The violet end product can be seen very well with the naked eye in this case. This assay was used, on the one hand, for rapid screening of FPLC fractions and, on the other hand, to demonstrate AlaDH activity in native protein gels.

The basis of this assay is a reaction mix consisting of 1/2 vol. of 0.5 M glycine·KOH, pH 10.2, and 1/8 vol. each of 0.5 M L-alanine, 6.25 mM NAD^+, 2.4 mM NBT and 0.64 mM PMS.

For the analysis of protein fractions the substrate mix was added 1:1 to the solution to be tested. Native gels were incubated directly in 10 ml of substrate mix after electrophoresis.

A positive reaction can be seen after 5 minutes at the latest.

2.4.2 Semiquantitative Assay

This assay was used to investigate AlaDH activities in mycobacteria.

The mycobacteria were grown on Löwenstein medium. Bacteria were taken from the slant agar tubes using an inoculating loop, resuspended in water and adjusted to a turbidity equivalent to a McFarland Standard No. 5. For separation of cell aggregates the suspensions were treated in an ultrasound bath for 10 minutes.

Reaction mix (see 2.4.1) was then added 1:1 to the cells and incubation was carried out at RT for 10 minutes. After centrifuging at 20,000 g for 2 minutes, the absorption of the supernatant was measured against the blank value.

A batch to which no L-alanine was added was used as the reference measurement. An absorption change of one unit per minute in this test corresponds approximately to an absorption change of three units per minute in the case of the quantitative assay (measurement at 340 nm, see 2.4.3).

2.4.3 Quantitative Assay

In this assay, the quantitative change in the NADH content was measured directly at 340 nm.

The standard reaction batches had a volume of 1 ml. The composition is shown in **Tab. 2.7.** The absorption was followed over a period of 10 minutes at 37°C and 340 nm. The extinction coefficient ε of NADH at 340 nm is 6.22×10^6 cm^2/mol.

The standard batches were varied as stated in the text in order to determine the biochemical properties of the enzyme. Every measured value shown represents the average value of at least two, but normally three, independent measurements.

An AlaDH unit is defined as the amount of enzyme that catalyses in one minute the formation of 1 µmol of NADH in the oxidative deamination reaction.

Tab. 2.7: Composition of the quantitative AlaDH assay

The composition of the reaction batch for the oxidative deamination is shown on the left and that for the reductive amination is shown on the right.

oxidative deamination	reductive amination
125 mM glycine·KOH, pH 10.2	1 M NH_4Cl/NH_4OH, pH 7.4
100 mM L-alanine	20 mM pyruvate
1.25 mM NAD^+	0.5 mM NADH

3. The distribution of alanine dehydrogenase within the mycobacteria

Both at the gene level and at the protein level, the next aspect to be investigated was in which mycobacteria an alanine dehydrogenase is present. Based on the virulence, the question here was whether the AlaDH activity correlates with that property.

3.1 *In vivo* AlaDH activity

Since AlaDH activity is the exception rather than the rule in the microbe world it was interesting to query whether that enzyme is ubiquitous within the mycobacteria or whether it is restricted to certain species and strains. Thereby, inferences can then be made in turn about questions such as:

Do AlaDH-producing strains have common features in their mode of life?

Does a specific method or phase of growth induce AlaDH production?

How does regulation of the AlaDH occur?

Can other metabolic routes replace the reaction catalysed by AlaDH?

What phenotype would *AlaDH* mutants have to exhibit?

All available strains were therefore investigated for production of AlaDH activity. The repertoire comprised a total of 44 mycobacterial strains, representing 29 different species. In addition, the two strains *Nocardia asteroides* and *Rhodococcus equi* which are closely related to the mycobacteria were tested.

In order for the activities measured in the test system to be compared with one another, all the bacterial suspensions were adjusted to a density corresponding to the turbidity of a McFarland Standard No. 5. At the time of measurement, the strains were in the late exponential phase.

In addition to the AlaDH measurement, a measurement was also carried out in which L-alanine was missing from the reaction batch. The activity of that batch is a measure of other NAD^+-reducing processes proceeding in parallel. The difference between that batch and the standard batch corresponds to the net AlaDH activity (ΔA_{595} value).

According to the activities measured the strains investigated can be divided into three groups. The first group is that of the strongly positive strains **(Tab. 3.1)**. Combined into that group are the strains that have an AlaDH activity of more than 0.5 ΔA_{595} units in the test system used.

Tab. 3.1: Strains having a strongly positive AlaDH activity

The way in which this assay was carried out is described in 2.4.2.

strain	AlaDH activity [ΔA_{595}]
M. marinum 3	2.327
M. chelonae 7	1.842
M. microti 1	0.919
M. tuberculosis $H37R_v$	0.592

Classified as strongly positive were the two strains that are pathogenic for fish, *M. chelonae* and *M. marinum*, and the two likewise pathogenic strains, *M. microti* and *M. tuberculosis* $H37R_v$, the latter being a virulent tuberculosis reference strain.

The second group, that of the moderately positive strains, comprises those having an activity between 0.1 and 0.5 ΔA_{595} units **(Tab. 3.2)**.

Tab. 3.2: *Strains having a moderately positive AlaDH activity*

The way in which this assay was carried out is described in 2.4.2.

strain	AlaDH activity [ΔA_{595}]	strain	AlaDH activity [ΔA_{595}]
M. smegmatis 3	0.375	*M. tuberculosis* 49	0.138
M. ulcerus 1	0.369	*M. tuberculosis* 130	0.118
M. africanum 1	0.287	*M. smegmatis* 1	0.116
M. tuberculosis 118	0.210	*M. tuberculosis* 132	0.111
M. tuberculosis 145	0.190	*M. tuberculosis* 146	0.111
M. intracellulare 1	0.155	*M. tuberculosis* 1	0.110

In this group, apart from *M. smegmatis*, only pathogenic, clinical isolates of *M. tuberculosis* and other mycobacteria are to be found. Both strains of *M. smegmatis* tested, however, also exhibit very high NAD^+-reducing activities in the absence of L-alanine. It is also important to mention at this point that the strain *M. smegmatis* 1-2c (a derivative of *M. smegmatis* mc^26; Zhang *et al.*, 1991; Garbe *et al.*, 1994; of Dr. Peadar Ó Gaora, St. Mary's Hospital, London), a strain for genetic studies in mycobacteria, does not exhibit any AlaDH activity, but likewise has a high background activity.

Finally, in the last group, there are listed all the strains found to be negative for AlaDH activity, that is to say that have an activity of less than 0.1 ΔA_{595} units **(Tab. 3.3)**.

Tab. 3.3: *Strains without AlaDH activity*

The way in which this assay was carried out is described in 2.4.2.

strain	AlaDH activity [ΔA_{595}]	strain	AlaDH activity [ΔA_{595}]
N. asteroides 1	0.048	*M. bovis* BCG 4	0.001
M. flavescens 1	0.042	*M. terrae* 2	0.001
M. tuberculosis H37R$_a$	0.032	*M. tuberculosis* 60	0
M. nonchromogenium 1	0.026	*M. tuberculosis* 163	0
M. fortuitum 11	0.022	*M. gastri* 1	0
M. asiaticum 1	0.021	*M. gordonae* 3	0
M. bovis BCG 2	0.013	*M. kansasii* 1	0
M. lufu 1	0.013	*M. parafortuitum* 1	0
R. equi 1	0.011	*M. peregrinum* 1	0
M. bovis 3	0.010	*M. phlei* 1	0
M. scrofulaceum 1	0.009	*M. phlei* 4	0
M. intracellulare 5	0.007	*M. scrofulaceum* 8	0
M.thermoresistibile 1	0.006	*M. simiae* 1	0
M. avium 1	0.002	*M. vaccae* 3	0
M. triviale 1	0.002	*M. xenopi* 7	0

This by far the largest group mainly comprises opportunistic and non-pathogenic strains, and also the two strains related to the mycobacteria, *Nocardia asteroides* and *Rhodococcus equi*. Exceptions were two clinical tuberculosis isolates and the pathogen of bovine Tb, *M. bovis*, but also the two vaccination strains of *M. bovis* BCG studied.

A graph of AlaDH activities in the realm of the mycobacteria is given in **Fig. 3.16**, ordered according to phylogenetic aspects.

The exact name of the individual strains is given in **Tab. 2.3.** The statements *fast-growing* and *slow-growing* should not be interpreted strictly but, rather, represent a tendency within the groups shown.

To summarise, the distribution of AlaDH activity within the world of the mycobacteria may be described as follows:

1 By far the highest activity is exhibited by the two strains that are pathogenic for fish, *M. chelonae* and *M. marinum*.

2 Within the strains of *M. tuberculosis* there is a tendency that, as virulence decreases, AlaDH activity also decreases ($H37R_v$ > clinical isolates > $H37R_a$).

3 All strains classified as positive are virulent. The only exception is *M. smegmatis* which, however, is very easily distinguishable on the basis of its high background activity.

4 Not all virulent strains are AlaDH-positive.

5 *M. tuberculosis* can be distinguished by means of AlaDH activity from the vaccination strain *M. bovis* BCG.

3.2 The gene for alanine dehydrogenase

3.2.1 The first PCR fragments

Having quantified the AlaDH activities within the various strains, the next question was why some strains produce the enzyme but others do not. The degree of expression also differs clearly in some cases, even between closely related types.

The absence of measurable activity can to a certain extent be explained by the fact that not all the strains were in exactly the same phase of growth, since it is very difficult to grow all strains parallel, at the same stage. A reason for the absence of activity might, however, also be that genetic changes have an effect on the expression of the gene. Those changes might have occurred in the coding or in the regulatory region.

In order to verify that fact, an attempt was made to amplify the *AlaDH gene* from various strains, completely or partially, by means of PCR. The primers used for this were oligonucleotides based on the sequence of *M. tuberculosis* $H37R_v$ (Andersen *et al.*, 1992; see Section 2.2.2, **(Tab. 2.5)**).

The primer pairs used to detect the AlaDH, the expected length of the respective products and the annealing temperatures of the PCR respectively used are summarised in **Tab. 3.4.**

Tab. 3.4: Primer pairs for the detection of AlaDH in mycobacteria.

The sequences of the primers are given in **Tab. 2.5.**

name	primer #1	primer #2	product	temperature
Annabel	AlaDH-F1	AlaDH-RM	433 bp	65°C
Beatrice	AlaDH-F1	AlaDH-R2	1102 bp	45°C
Claudette	AlaDH-F1	AlaDH-R3	1120 bp	55°C
Désirée	AlaDH-F1	AlaDH-R6	1072 bp	45°C
Eleonore	AlaDH-F1+	AlaDH-R1	1099 bp	55°C
Francoise	AlaDH-F1+	AlaDH-R2	1117 bp	50°C
Giselle	AlaDH-F2	AlaDH-R7	757 bp	35°C
Helen	AlaDH-F4	AlaDH-RM	1080 bp	55°C
Isabelle	AlaDH-F4	AlaDH-R6	1050 bp	55°C
Jeanette	AlaDH-F5	AlaDH-R1	507 bp	45°C
Karen	AlaDH-F5	AlaDH-R4	834 bp	45°C
Larissa	AlaDH-F6	AlaDH-R4	786 bp	55°C
Melanie	AlaDH-F6	AlaDH-R5	405 bp	55°C

The first attempts to detect the gene for AlaDH in various mycobacterial species were made with the primer pair *Annabel*. The result obtained in this case was somewhat surprising. All of the strains of the *M. tuberculosis* complex exhibited the expected 433 bp fragment. In addition, in all of these strains, an additional fragment of approximately 900 bp had been amplified **(Fig. 3.17)**.

PCR of various strains using the primer pair *Annabel*.

In these PCRs, 40 cycles having the following sequence were used in each case: *melting* 2 min at 96°C, *annealing* 2 min at 65°C and *extension* 3 min at 72°C. The $MgCl_2$ concentration was 1.5 mM.

track 1:	*M. tuberculosis* $H37R_v$	track 6:	*M. bovis* BCG 4
track 2:	*M. tuberculosis* $H37R_a$	track 7:	*M. africanum*
track 3:	*M. tuberculosis* 1	track 8:	*M. microti* 1
track 4:	*M. bovis* 3	track 9:	*M. marinum* 3
track 5:	*M. bovis* BCG 2	track 10:	*M. chelonae* 7

As was to become apparent, that second fragment was also a part of the *AlaDH gene*, which had come into being as a result of the binding of the primer AlaDH-RM to a site located closer to the C-terminus. By increasing the annealing temperature in the PCR from 65 to 69°C it was possible to suppress that second fragment (see **Fig. 3.18**, tracks 2 and 3).

What was actually astounding, however, was the appearance of the amplified fragment in all the strains of the *M. tuberculosis* complex, irrespective of the existence of AlaDH activity.

In the case of a number of other strains also, it was possible to amplify one or more fragments using the primer pair *Annabel*. The amplified bands were not, however, particularly strong in most cases and, in view of the 40 PCR cycles, they may therefore be regarded as background. Presumably, weak unspecific reactions are involved. However, the possibility that the PCR primers were unable to bind optimally to the target sequence owing to insufficient homology between the various species also cannot be excluded.

The two fish pathogen strains having a strong AlaDH activity, *M. marinum* and *M. chelonae*, exhibited distinctly different behaviours in the PCR with the primer pair *Annabel*. Whereas *M. marinum* yielded a product of approximately 540 bp, no fragment could be obtained in the case of *M. chelonae* under the chosen conditions with the primer pair *Annabel* (**Fig. 3.17**, tracks 9 and 10).

3.2.2 The *AlaDH gene* of the *M. tuberculosis* complex

Since the presence of the gene for AlaDH had been detected in all the strains of the *M. tuberculosis* complex, the question was how to explain the discrepancy with the measured activities.

For that reason, amplification of larger fragments of the gene was begun. Of *M. tuberculosis* $H37R_v$ all the fragments listed in **Tab. 3.14** could be amplified (some of those fragments are shown in **Fig. 3.18**). Of the other strains of the *M. tuberculosis* complex all the PCR reactions from **Tab. 3.15** that were tested likewise proceeded positively. Every reaction was not, however, replicated with every strain.

PCR products of the strain *M. tuberculosis* $H37R_v$

In these PCRs, 40 cycles were used in each case as shown in **Fig. 3.17**. With the exception of tracks 2 and 3, the annealing temperatures are given in **Tab. 3.14**. The $MgCl_2$ concentration in the case of the primer pair *Annabel* was 1.5 mM, and that in all the other reactions was 3 mM.

track 1: KBL	track 7: *Giselle*
track 2: *Annabel, 65°C*	track 8: *Helen*
track 3: *Annabel, 69°C*	track 9: *Isabelle*
track 4: *Désirée*	track 10: *Larissa*
track 5: *Eleonore*	track 11: *Melanie*
track 6: *Francoise*	track 13: KBL

The amplified region of all the strains of the *M. tuberculosis* complex comprises 1260 bp. It contains the complete coding segment for the AlaDH, and a further 75 bp upstream and 63 bp downstream. This region of all the strains of the *M. tuberculosis* complex was sequenced completely **(Fig. 3.19)**. Only in the last 20 bases or so did inaccuracies creep in. The complete remaining region has, however, been confirmed by repeated sequencing.

It can be ascertained that all the sequences are identical to the published sequence of the λAA65 clone (Andersen *et al.*, 1992) apart from three sites.

Alignment of the AlaDH gene and the flanking regions of various strains of the *M. tuberculosis* complex

The line designated "40 kD" gives the sequence of Andersen *et al.* (1992). Sequence differences are each marked with a "*" above the sequence. The start and stop codons are also marked above the sequence. The bases printed in bold typeface at the end of the sequence are sequencing inaccuracies.

The first site at which the sequences differ is base -32, that is to say upstream of the translation start signal. Interestingly, the sequences of *M. tuberculosis* $H37R_v$ and $H37R_a$ determined in this study differ from the sequence of Andersen and co-workers (Andersen *et al.*, 1992) at that site. All the other

sequences investigated in this study, including that of the third strain of *M. tuberculosis* tested, agree with the sequence of Andersen.

This is astonishing, given that the originally published sequence is based on the clone of a λgt11 bank that had been produced from the strain *M. tuberculosis* $H37R_v$. The question of whether an error had perhaps been introduced by the PCR was therefore investigated. That, however, did not prove to be correct. It might also be possible, however, that the strain of *M. tuberculosis* $H37R_v$ used in this study had a different origin from that of Andersen. Similar small variations are also known in the case of various *M. bovis* BCG strains of different origins.

At the second site, all strains of the *M. tuberculosis* complex differ from the published sequence of the AlaDH of *M. tuberculosis* $H37R_v$. The region concerned is that of bases 38 to 49. Within those twelve bases the sequence AATTCC is repeated; bases 44 to 49, therefore, represent a *direct repeat* of bases 38 to 43. In all eight of the strains sequenced, that pattern is to be found, however, only once in each. It is therefore to be assumed that a sequencing or reading error has crept in in the case of the sequence determined by Andersen *et al.* (1992). As a result, the gene sequence and the amino acid sequence derived therefrom changes as follows:

Andersen *et al.*, 1992:

gene sequence	A	A	C G	A A	T T	C C	A A T T C C G G G T G
protein sequence	Asn		Glu	Phe	Gln	Phe	Arg Val

This study:

gene sequence	A	A	C G	A A	T T	C	- - - - - - C G G G T G
protein sequence	Asn		Glu	Phe	-	-	Arg Val

What is effectively involved, therefore, is the "loss" of the two amino acids glutamine and phenylalanine. After that deletion, the sequence continues as published by Andersen *et al.* (1992).

That fact was confirmed by N-terminal sequencing of the protein. Neither in the native protein of *M. tuberculosis* $H37R_v$ nor in the recombinant protein from *E. coli* were the two amino acids to be found.

The third site that differs is base 272. At that site, with the exception of three strains, there is an adenine residue. In the case of those three strains, *M. bovis* and two strains of *M. bovis* BCG, that base has been deleted. The deletion leads to a reading frame shift that affects the entire following part of the resulting protein. As a result of that reading frame shift, an opa1 stop signal occurs at bases 404 to 406. The product of that gene is therefore only about one third the size of the functional AlaDH of the other strains.

What is decisive in the case of this third discrepancy in the gene sequence is the fact that it occurs in precisely the three strains that do not exhibit any AlaDH activity. *M. bovis* and *M. bovis* BCG are the only strains of the *M. tuberculosis* complex that do not exhibit any activity. All the other

strains were classified as being moderately or strongly positive. The observed deletion, therefore, is the reason for the absence of a functional AlaDH. Since, however, the truncated protein also could not be detected with the mAb HBT-10 (the epitope of HBT-10 lies in the region before the reading frame shift), it is to be assumed that the truncated protein is not produced in the first place or is produced only in very small amounts that are not detectable with the mAb HBT-10.

4 AlaDH activity and AlaDH gene in mycobacteria

AlaDH activity in mycobacteria. The AlaDH activities measured permit a number of interesting observations regarding the mode of life of the organisms that have a positive activity.

The strains that have a strong activity are all pathogenic. It is interesting here that two of the four strains falling into that group are pathogenic for fish (Austin & Austin, 1987). Both of those, *M. marinum* and *M. chelonae*, can, however, infect humans also (Wallace *et al.*, 1983; Johnston & Izumi, 1987). In contrast to tuberculosis, however, they cause morbid infections of the upper layers of the skin in most cases, which are relatively unproblematical to treat in most cases.

M. chelonae is a comparatively fast-growing, non-chromogenic bacterium. Infections in humans often occur in the form of secondary wound infections following operations (Cooper *et al.*, 1989). *M. marinum* is a slow-growing organism that forms a yellow pigment when growing in light. Infections with *M. marinum* have been detected in more than 50 poikilothermic species (reptiles, amphibians, fish). In humans, the bacterium usually manifests itself in the elbow or knee area.

The two other strains having a strongly positive AlaDH activity

are representatives of the *M. tuberculosis* complex. They are the tuberculosis reference strain, *M. tuberculosis* $H37R_v$, and the strain *M. microti*, which is regarded as a phylogenetic link between *M. tuberculosis* and *M. bovis*.

With the exception of *M. smegmatis*, all of the strains classified as moderately positive also are pathogenic. The majority of those strains comprises clinical isolates of *M. tuberculosis*. Pathogenic variants of tuberculosis strains appear, therefore, to have AlaDH activity as a rule. Two isolates were also found, however, that did not exhibit any AlaDH activity. The only non-pathogenic organism having AlaDH activity is the fast-growing strain *M. smegmatis*. *M. smegmatis* is characterised, however, by an unusually high NAD^+-reducing background activity and is therefore very easily distinguished from all the other strains having AlaDH activity. Furthermore, in the strain *M. smegmatis* 1-2c, a mycobacterial expression strain, no AlaDH activity was found.

Within the 44 mycobacteria strains tested, and that is by far the majority of all known strains, the following conclusion is therefore permissible:

⇒ a slow-growing mycobacterium having positive AlaDH activity is virulent.

The converse of that statement is, however, false. Among the strains that do not have AlaDH activity, several are virulent. Nevertheless, one cannot help finding a tendency, although not strong, for AlaDH activity to increase with increasing pathogenicity of a strain. That thesis is lent greater weight especially by the activities of the various strains of *M. tuberculosis*. By far the highest activity is exhibited by the strain $H37R_v$, which serves as the reference strain for all tubercul-

osis laboratories and which is known to be highly infectious. At the very end of the scale there is the avirulent derivative of $H37R_v$, the strain $H37R_a$. Ranged between those two poles are the clinical tuberculosis isolates, some of which exhibit slightly more activity and some slightly less.

The *AlaDH gene* in mycobacteria. The gene for alanine dehydrogenase could be identified in all the strains of the *M. tuberculosis* complex investigated and in the strain *M. marinum*.

The decisive point when comparing the sequences within the *M. tuberculosis* complex is the deletion of base 272 which, in the case of the strains of *M. bovis* and *M. bovis* BCG investigated, result in a reading frame shift and ultimately in a truncated, non-functional protein. In the case of those strains, no AlaDH activity could be detected in cell extracts either. Those data also agree with the results of Andersen *et al.* (1992) who obtained signals with those strains in Southern blots but could not detect any protein in Western blots.

By amplifying and sequencing the gene it was possible in this study to find the reason for this. It is also necessary to take into consideration, however, that other changes in the regulatory gene segments may be responsible for the absence of the truncated protein. This might be a measure taken by the cell not to invest energy in a protein that is not capable of functioning. In general, not much is known yet about regulatory gene sequences in mycobacteria (Dale & Patki, 1990; Gupta *et al.*, 1993). It appears, however, that, in accordance with the principle of *enhancers*, segments located further away may also have a not inconsiderable influence on the gene expression. The mutations required for a regulation of the production of the protein do not necessarily have to lie, therefore,

in the region sequenced in this study.

The other *AlaDH gene* identified, that of *M. marinum*, is clearly different at the DNA level from the genes of the *M. tuberculosis* complex. Nevertheless, four of five bases (80.4%) are, however, still identical on average upon comparison of those sequences. That value is even higher at the protein level (85.3% identity, 92.0% similarity). Since, however, AlaDH activity has also been found in a number of other species, it is to be assumed that the corresponding genes could not be amplified under the conditions used for lack of homology to the primers used. A more detailed study with regard to that point should be able to find those genes also. A comparison of all those sequences might allow further conclusions to be drawn on the role of the enzyme.

It is furthermore conceivable that, using such a sequence comparison, it should be possible to develop a PCR process with which mycobacteria that have an *AlaDH gene* can be distinguished from one another. And, as it has been possible to show in this study, it is precisely the strains that are of importance to humans that possess an *AlaDH gene*. Especially the possibility of being able to distinguish the pathogen *M. tuberculosis* from the vaccination strain *M. bovis* BCG using such a PCR assay makes such a project appear interesting.

Prospects. The 40 kD antigen with which this study has been concerned is a worthwhile subject for more detailed investigations in several respects. One aspect that has not been considered in detail in this study is the possible use of that enzyme in medical diagnostics. For example, assays that are based on an AlaDH have already been described for the enzymes dipeptidase (Ito *et al.*, 1984), γ-glutamyltransferase (Kondo *et al.*, 1992) and γ-glutamyl cyclotransferase (Takahashi *et*

al., 1987). All three of the enzymes mentioned are to be found in altered urine, serum and/or blood concentrations in various diseases.

The main attention, however, is on the use of the 40 kD antigen in the case of tuberculosis. Several points from which this can be approached are conceivable.

In diagnostics alone, it is possible to envisage several possible ways in which the 40 kD antigen or its underlying gene might be used. Since the recombinant protein can easily be recovered from the overproducing *E. coli* strain, it appears worthwhile to study the usefulness of that protein in serology. In addition, it might be possible to develop diagnostic processes based on the direct detection of AlaDH activity or, as already mentioned, on amplification of specific parts of the gene. The deletion of base 272 in the strains *M. bovis* and *M. bovis* BCG may serve here as the starting point for discrimination of those two strains from *M. tuberculosis*.

It also should be possible to create a PCR assay for the strain *M. marinum* which, of course, at the gene level, differs not inconsiderably from the *M. tuberculosis* complex. Up to now, a PCR assay relying on amplification of a part of the gene sequence coding for the 16S rRNA has been used for that purpose (Knibb *et al.*, 1993). This is of great importance in view of the increasing number of infections with *M. marinum* in fish farms in recent years. Infections in humans also have been reported more frequently in recent years (Harris *et al.*, 1991; Kullavanijaya *et al.*, 1993; Slosarek *et al.*, 1994).

The observation that the virulence of a strain of *M. tuberculosis* correlates very well with its AlaDH activity again poses the question whether the enzyme represents a virulence factor.

To answer that question, approaches such as knock-out of the gene in *M. tuberculosis* or overexpression of the gene in a strain of low virulence are conceivable. In both cases, the virulence can be tested in an animal model.

The disclosure also includes all conceivable combinations of the individual features disclosed.

6. Appendices

List of abbreviations

A	pre-exponential factor or impact factor
A_{xxx}	absorption at a wavelength of xxx nm
AlaDH	L-alanine dehydrogenase (E.C. 1.4.1.1.)
AMC	Academic Medical Centre, Amsterdam, The Netherlands
Ap	ampicillin
AP	alkaline phosphatase
app.	apparent
AS	amino acid
ATCC	American Type Culture Collection, Rockville, USA
ATP	adenosine triphosphate
BCG	Bacille Calmette Guérin
BCIG	5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside
BCIP	5-bromo-4-chloro-3-indolyl phosphate
Boc	tert-butoxycarbonyl
bp	base pair(s)
cfu	colony forming units
Cm	chloramphenicol
Conc	concentration
DMEM	Dulbecco's Modified Eagle Medium
DMF	dimethylformamide
DMSO	dimethyl sulphoxide
DNA	deoxyribonucleic acid
DTNB	dithiobisnitrobenzoic acid
DTT	dithiothreitol
E_a	activation energy
EDTA	ethylenediamine tetraacetate
Eth	ethionamide

F	farad
f.a.	for analysis, of the highest degree of purity
FBS	foetal bovine serum
FCS	foetal calf serum
Fmoc	9-fluorenylmethoxycarbonyl
FPLC	Fast Protein Liquid Chromatrography
frag.	fragment
g	acceleration due to gravity
GBF	Gesellschaft für biotechnologische Forschung mbH, Braunschweig, Germany
GlcNAc	N-acetylglucosamine
Gm	gentamicin
GOGAT	glutamine oxoglutarate aminotransferase
GS	glutamine synthetase
GST	glutathione S-transferase
h	hour(s)
HBSS	Hank's Balanced Salt Solution
HIV	Human Immunodeficiency Virus
HOBt	hydroxybenzotriazole
HRP	horseradish peroxidase
Hsp	heat shock proteins
Ig	immunoglobulin
IL	interleukin
INH	isonicotinic acid hydrazide, isoniazide
IPTG	isopropyl-β-D-thiogalactoside
k	conversion rate of an enzyme
kb	kilobases
KBL	kilobase ladder
kD, kDa	kilodalton

KIT	Royal Tropical Institute, Amsterdam, The Netherlands
K_M	Michaelis constant
Km	kanamycin
MΦ	macrophage(s)
mAb	monoclonal antibody
MAIS	*M. avium - M. intracellulare - M. scrofulaceum* complex
MBP	maltose binding protein
MCAC	metal chelate affinity chromatography
mesoDAP	meso-diaminopimelic acid
min	minute(s)
m.o.i.	multiplicity of infection
MRC	Medical Research Council, Tuberculosis and Related Infections Unit, London, England
MTT	thiazolylblue tetrazolium bromide
MurNAc	N-acetylmuramic acid
MurNG1	N-glycolylmuramic acid
NAD^+	nicotinamide adenine dinucleotide, oxidised form
NADH	nicotinamide adenine dinucleotide, reduced form
$NADP^+$	nicotinamide adenine dinucleotide phosphate, oxidised form
NADPH	nicotinamide adenine dinucleotide phosphate, reduced form
n.d.	not determined
NBT	nitroblue tetrazolium chloride
No.	number
NTP	any nucleotide in the form of a triphosphate
oD	oxidative deamination
ON	overnight
ORF	open reading frame
OtBu	tert-butyl ester

PAGE	polyacrylamide gel electrophoresis
pac	protein antigen c, old term for the 40 kD antigen
PCR	polymerase chain reaction
Pfp	pentafluorophenyl
PMA	phorbol myristate acetate
Pmc	pentamethylchromane
PMS	phenazine methosulphate
PNT	pyridine nucleotide transhydrogenase
PPD	purified protein derivative
PVDF	polyvinylidene difluoride
R	Rydberg constant or resistance (when superscript letter)
rA	reductive amination
rec	recombinant
Rha	rhamnose
Rif	rifampicin
RIV	National Institute of Public Health and the Environment, Buthoven, The Netherlands
RNA	ribonucleic acid
RNI	reactive nitrogen intermediates
ROI	reactive oxygen intermediates
rpm	revolutions per minute
rRNA	ribosomal ribonucleic acid
RT	room temperature
SDS	sodium dodecyl sulphate
sec	second(s)
Str	streptomycin
Tb	tuberculosis
TEMED	N,N,N',N'-tetramethylethylenediamine
TIR	translation initiation region

Tris	tris(hydroxymethyl)aminomethane
Trt	trityl
ts	temperature-sensitive
Tween	polyoxyethylenesorbitan monolaurate
U	unit(s)
v_{max}	maximum reaction velocity
VMDC	Veterinary Microbiological Diagnostic Centre, Utrecht, The Netherlands
vol.	volume
WHO	World Health Organisation
WKZ	Academisch Ziekenhuis, Utrecht, The Netherlands

Abbreviations for amino acids and nucleotides

amino acid	3-letter code	1-letter code
alanine	Ala	A
arginine	Arg	R
asparagine	Asn	N
aspartate	Asp	D
cysteine	Cys	C
glutamine	Gin	Q
glutamate	Glu	E
glycine	Gly	G
histidine	His	H
isoleucine	Ile	I
leucine	Leu	L
lysine	Lys	K
methionine	Met	M
phenylalanine	Phe	F
proline	Pro	P
serine	Ser	S
threonine	Thr	T
tryptophan	Trp	W
tyrosine	Tyr	Y
valine	Val	V

base	**nucleoside / nucleotide**	**abbreviation**
adenine	adenosine	A
cytosine	cytidine	C
guanine	guanosine	G
uracil	uridine	U
thymine	thymidine	T

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PATENT CLAIMS

1. An enzymatic test kit for the diagnosis of tuberculosis and other mycobacterial infections in humans and animals by determination of the activity of alanine dehydrogenase (E.C. 1.4.1.1), comprising L-alanine, nicotinamide adenine dinucleotide (oxidised form; NAD^+), phenazine methosulphate (PMS) and nitroblue tetrazolium chloride (NBT).

2. A method for the diagnosis of tuberculosis and other mycobacterial infections of humans and animals, ***characterised*** in that the activity of alanine dehydrogenase (E.C. 1.4.1.1.) is measured with an enzymatic test kit according to claim 1.

3. A method according to claim 2, ***characterised*** in that
(i) possible tuberculosis pathogens, such as *M. tuberculosis*, are isolated,
(ii) a crude cell extract is made,
(iii the extract is incubated in solution and
(iv) the absorption is measured.

4. A method according to claim 2 and/or 3, ***characterised*** in that clinical samples, such as body fluids, are subjected directly to tuberculosis diagnosis and the alanine dehydrogenase activity is measured.

5. A method according to claim 2, ***characterised*** in that cells, strains and/or species of disease-causing organisms (mycobacteria) are differentiated from non-virulent cells and strains.

6. A method according to claim 5, ***characterised*** in that cells, strains and/or species of disease-causing organisms of the *M. tuberculosis* complex are identified and differentiated.

7. A method according to any one of the preceding claims, ***characterised*** in that the method is carried out in the presence of substances that inhibit tuberculosis and other mycobacterial infections of humans and animals and those inhibiting substances are optionally recovered.

8. A method according to any one of the preceding claims, ***characterised*** in that it is carried out

(i) to control epidemics and/or

(ii) after vaccinations (vaccination follow-up) in humans and animals.

9. A DNA sequence selected from the following group or other partial sequences of the alanine dehydrogenase gene of *M. tuberculosis* (Fig. 2.5):

Name	Sequence	Orientation
AlaDH-F1	5'-ATGCGCGTCGGTATTCCG-3'	forward
AlaDH-F1+	5'-GCGCGTCGGTATTCCGACCG-3'	forward
AlaDH-F2	5'-GAGACCAAAACAACGAA-3'	forward
AlaDH-F4	5'-GAATTCCCATCAGCAATCTTGCAGA-3'	forward
AlaDH-F5	5'-GCCCCGATGAGCGAAGTC-3'	forward
AlaDH-F6	5'-GGGGCCGTCCTGGTGCC-3'	forward
AlaDH-F7	5'-GACGTCGACCTACGCGCTGAC-3'	forward
AlaDH-R1	5'-CTCGGTGAACGGCACCCC-3'	reverse
AlaDH-R2	5'-GGCCAGCACGCTGGCGGG-3'	reverse
AlaDH-R3	5'-CACCCGTTCGGACAGTAA-3'	reverse
AlaDH-R4	5'-CGCGGCCGACATCATCGC-3'	reverse
AlaDH-R5	5'-GGCCGACATCATCGCTTCCC-3'	reverse
AlaDH-R6	5'-CGAGACTAATTTGGGTGCCTTGGC-3'	reverse
AlaDH-R7	5'-ATTTGGGTGCCTTGGC-3'	reverse
AlaDH-RM	5'-GGCGGCGAGTCGACCGGC-3'	reverse

and partial sequences thereof and sequences that are hybridisable therewith preferably at a temperature of at least 20°C and especially at a concentration of 1M NaCl and a temperature of at least 25°C, for the diagnosis of tuberculosis and other mycobacterial infections in humans and animals.

10. The use of a DNA sequence according to claim 9 for the diagnosis of tuberculosis and other mycobacterial infections in humans and animals.

11. A method according to claim 10, ***characterised*** in that a DNA sequence according to claim 9 is used

(i) for hybridisation,

(ii) for culture confirmation of isolated strains and/or

(iii) for chromosomal fingerprinting, and cells, strains and/or types of mycobacteria are determined and differentiated and/or are used for the diagnosis of mycobacterial infections.

12. A method according to claim 10 or 11, ***characterised*** in that cells, strains and/or species of virulent mycobacteria are differentiated from non-virulent cells, strains and/or species.

13. A method according to claim 10, ***characterised*** in that cells, strains and/or species of the *M. tuberculosis* complex and other mycobacteria

(i) are isolated,

(ii) crude or purified genomic DNA or RNA is recovered,

(iii) a fragment that is identical or virtually identical to the sequence of the alanine dehydrogenase gene of *M. tuberculosis* (Fig. 2.3) is identified, preferably by amplification using a DNA sequence according to claim 9 as a primer sequence, after which digestion is carried out with a restriction enzyme, especially Bg1II, and gel

electrophoresis of the digested amplified DNA is carried out and/or the DNA sequence of the amplified DNA is determined.

14. A method according to claim 2 and/or 10, ***characterised*** in that a clinical sample is used directly and diagnosed for tuberculosis in humans and animals.

15. A method according to claim 2 and/or 10, ***characterised*** in that the method is carried out in the presence of substances that inhibit tuberculosis or mycobacterial infections of humans and animals and inhibiting substances determined are recovered or made.

16. A method according to claim 10, ***characterised*** in that it is used
(i) in antimycobacterial chemotherapy,
(ii) in the control of epidemics and/or
(iii) after vaccinations (vaccination follow-up) in humans and animals.

ABSTRACT

Tuberculosis is an infectious disease which kills more than three million people every year. Although both a vaccine and various methods of diagnosis and treatment are available, the efficacy of these measures is in urgent need of improvement given that the number of new cases is once again on the increase. Research focuses, among other things, on the characterization of antigens secreted in the early stages of the infection as they constitute the first point of contact of the immune system with the pathogen. The 40 KD-antigen described herein is present *in vivo* as a hexamer and, despite its high molecular weight and lack of a signal sequence, is present extracellularly after only a few days of growth. Functionally, it is an L-alanine dehydrogenase and reacts with the monoclonal antibody HBT-10 directed against this protein. HBT-10 was the first known antibody specific to a protein of *M. tuberculosis* which did not cross-react with the vaccine strain *M. bovis* BCG.

pJLA604Not

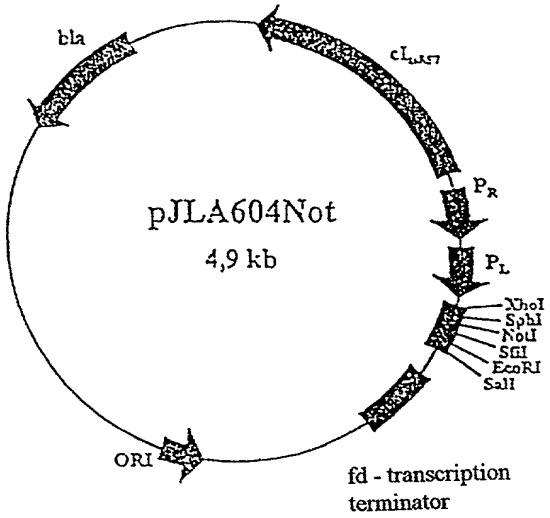

Fig. 2.1

pMSK12

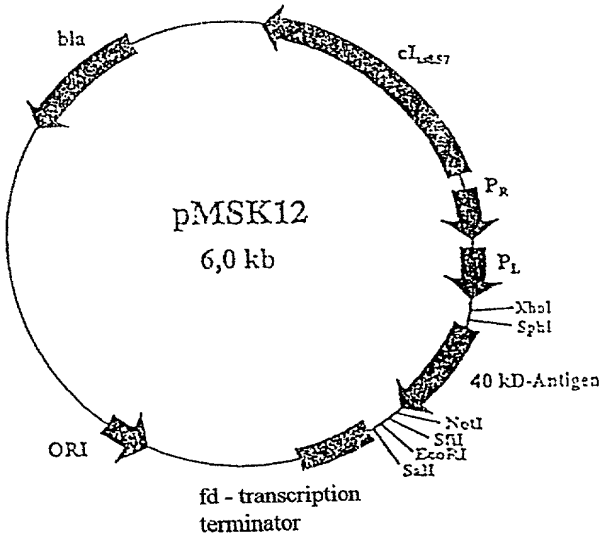

Fig. 2.2

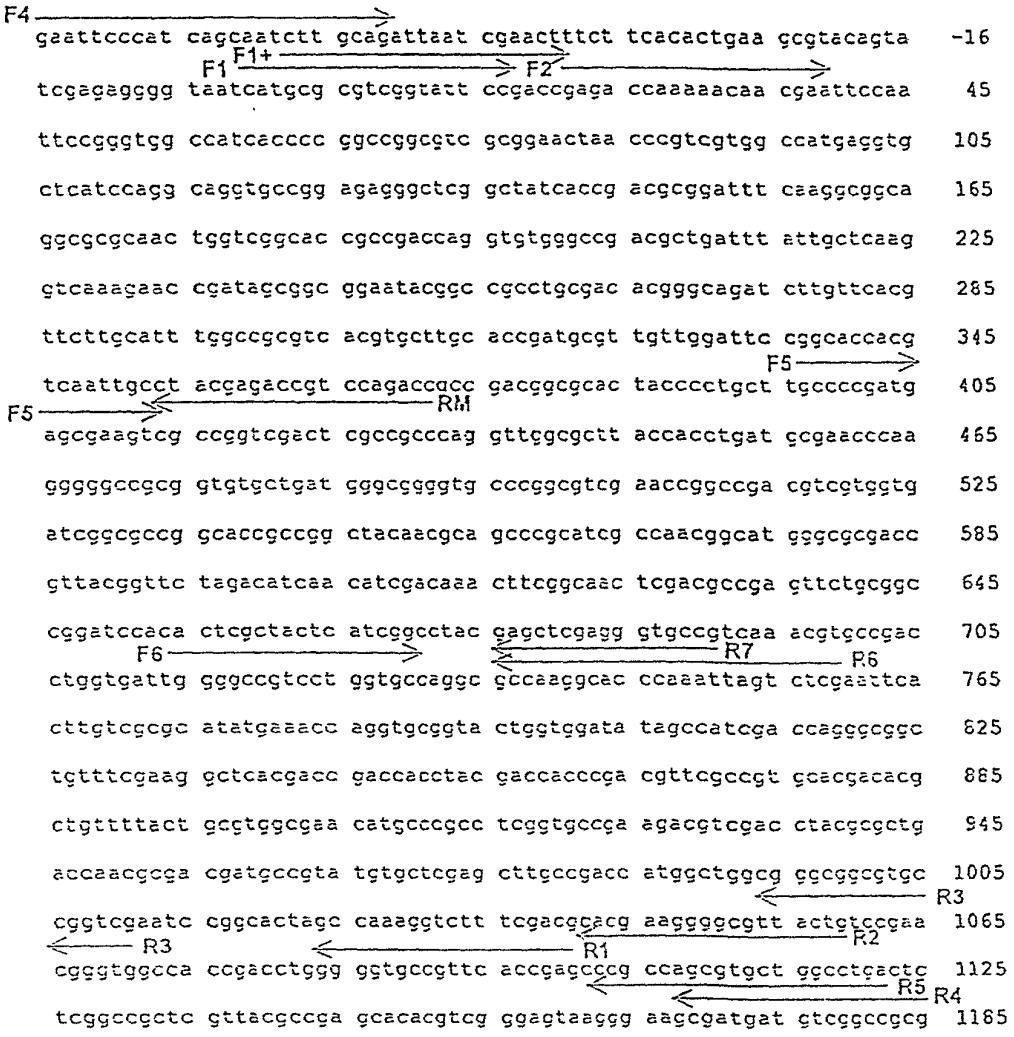

Fig. 2.3

Name	Sequence	Orientation
AlaDH-F1	5'-ATGCGCGTCGGTATTCCG-3'	forward
AlaDH-F1+	5'-GCGCGTCGGTATTCCGACCG-3'	forward
AlaDH-F2	5'-GAGACCAAAAACAACGAA-3'	forward
AlaDH-F4	5'-GAATTCCCATCAGCAATCTTGCAGA-3'	forward
AlaDH-F5	5'-GCCCCGATGAGCGAAGTC-3'	forward
AlaDH-F6	5'-GGGGCCGTCCTGGTGCC-3'	forward
AlaDH-F7	5'-GACGTCGACCTACGCGCTGAC-3'	forward
AlaDH-R1	5'-CTCGGTGAACGGCACCCC-3'	reverse
AlaDH-R2	5'-GGCCAGCACGCTGGCGGG-3'	reverse
AlaDH-R3	5'-CACCCGTTCGGACAGTAA-3'	reverse
AlaDH-R4	5'-CGCGGCCGACATCATCGC-3'	reverse
AlaDH-R5	5'-GGCCGACATCATCGCTTCCC-3'	reverse
AlaDH-R6	5'-CGAGACTAATTTGGGTGCCTTGGC-3'	reverse
AlaDH-R7	5'-ATTTGGGTGCCTTGGC-3'	reverse
AlaDH-RM	5'-GGCGGCGAGTCGACCGGC-3'	reverse

Fig. 2.5

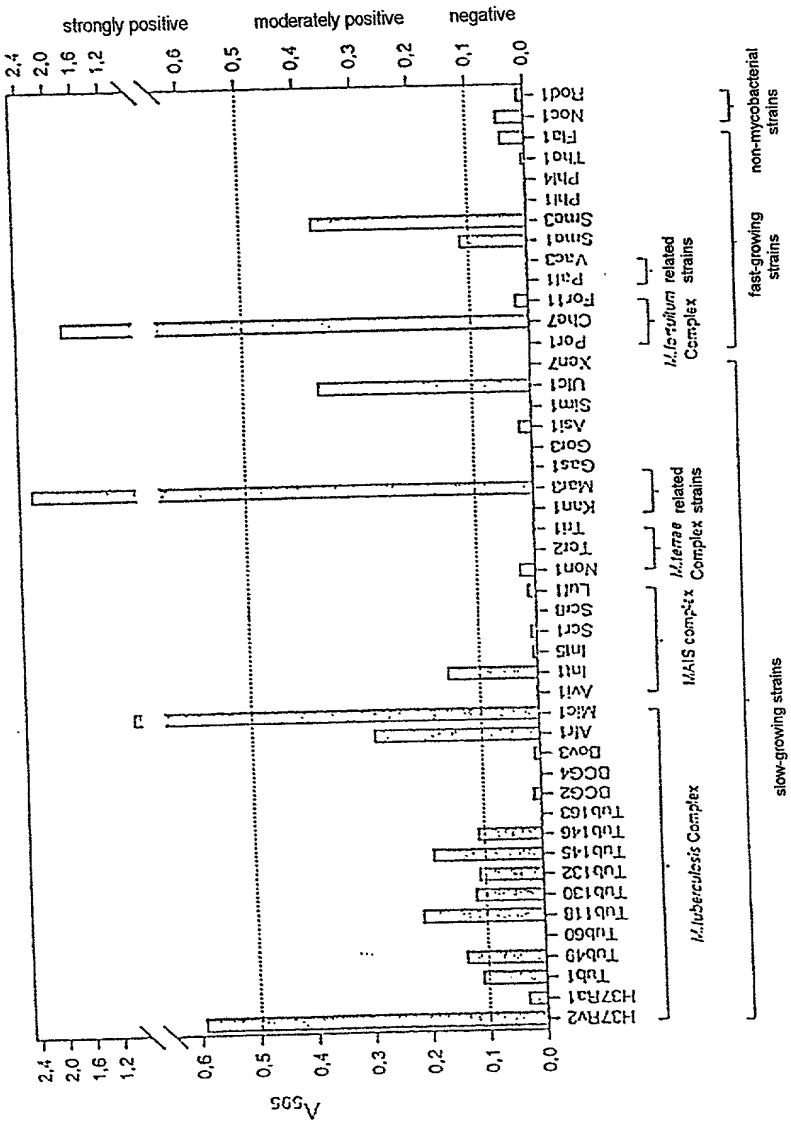
strongly positive
moderately positive
negative
2,4
2,0
1,6
1,2
0,6
0,5
0,4
0,3
0,2
0,1
0,0
Λ_{505}
Rod1
Noc1
Fla1
Tha1
Phl4
Phl1
Sme3
Sme1
Vac3
Pal1
For11
Chr7
Por1
Xen7
Ulc1
Sim1
Asi1
Gor3
Gas1
Mar3
Kan1
Tri1
Tor2
Non1
Lut1
Scr8
Scr1
Ins5
Int1
Avi1
Mic1
Afr1
Dov3
BCG4
BCG2
Tub63
Tub46
Tub45
Tub32
Tub30
Tub18
Tub60
Tub49
Tub1
H37Ra1
H37Rv2
M.tuberculosis Complex
MAIS complex
M.terrae related Complex strains
M.avium related Complex strains
slow-growing strains
fast-growing strains
non-mycobacterial strains

Fig. 3.16

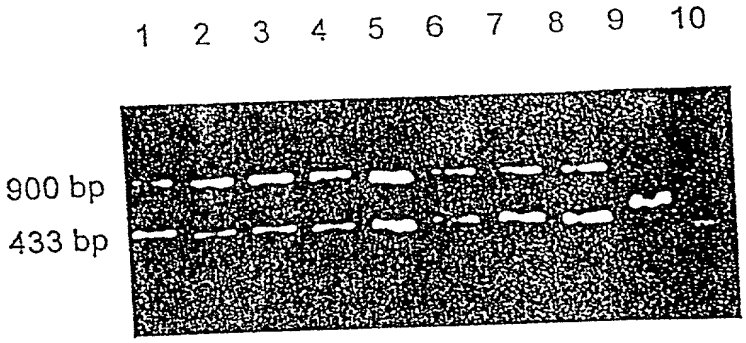

Fig. 3.17

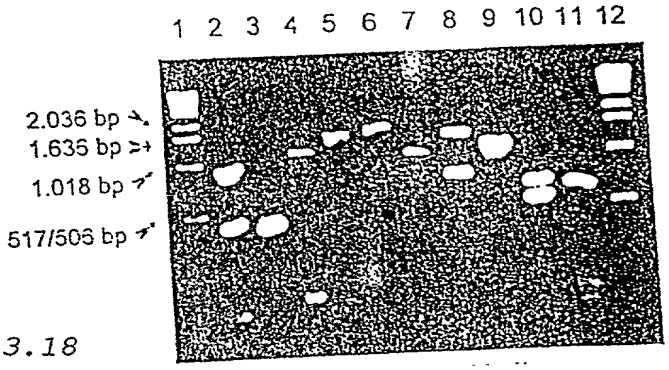

Fig. 3.18

Fig. 3.19

```
                                                 *
40kD  -60 ATCTTGCAGA  TTAATCGAAC  TTTCTTCACA  CTGAAGCGTA  CAGTATCGAG  AGGGGTAATC   -1
Tub1  -60 ATCTTGCAGA  TTAATCGAAC  TTTCTTCACA  CTGAAGCGTA  CAGTATCGAG  AGGGGTAATC   -1
H37Rv -60 ATCTTGCAGA  TTAATCGAAC  TTTCTTCATA  CTGAAGCGTA  CAGTATCGAG  AGGGGTAATC   -1
H37Ra -60 ATCTTGCAGA  TTAATCGAAC  TTTCTTCATA  CTGAAGCGTA  CAGTATCGAG  AGGGGTAATC   -1
BCG4  -60 ATCTTGCAGA  TTAATCGAAC  TTTCTTCACA  CTGAAGCGTA  CAGTATCGAG  AGGGGTAATC   -1
BCG2  -60 ATCTTGCAGA  TTAATCGAAC  TTTCTTCACA  CTGAAGCGTA  CAGTATCGAG  AGGGGTAATC   -1
Bov3  -60 ATCTTGCAGA  TTAATCGAAC  TTTCTTCACA  CTGAAGCGTA  CAGTATCGAG  AGGGGTAATC   -1
Afr1  -60 ATCTTGCAGA  TTAATCGAAC  TTTCTTCACA  CTGAAGCGTA  CAGTATCGAG  AGGGGTAATC   -1
Mic1  -60 ATCTTGCAGA  TTAATCGAAC  TTTCTTCACA  CTGAAGCGTA  CAGTATCGAG  AGGGGTAATC   -1
-----------------------------------------------------------------------------------
                                                    ******
          Start
40kD    1 ATGCGCGTCG  GTATTCCGAC  CGAGACCAAA  AACAACGAAT  TCCAATTCCG  GGTGGCCATC   60
Tub1    1 ATGCGCGTCG  GTATTCCGAC  CGAGACCAAA  AACAACG---  ---AATTCCG  GGTGGCCATC   60
H37Rv   1 ATGCGCGTCG  GTATTCCGAC  CGAGACCAAA  AACAACG---  ---AATTCCG  GGTGGCCATC   60
H37Ra   1 ATGCGCGTCG  GTATTCCGAC  CGAGACCAAA  AACAACG---  ---AATTCCG  GGTGGCCATC   60
BCG4    1 ATGCGCGTCG  GTATTCCGAC  CGAGACCAAA  AACAACG---  ---AATTCCG  GGTGGCCATC   60
BCG2    1 ATGCGCGTCG  GTATTCCGAC  CGAGACCAAA  AACAACG---  ---AATTCCG  GGTGGCCATC   60
Bov3    1 ATGCGCGTCG  GTATTCCGAC  CGAGACCAAA  AACAACG---  ---AATTCCG  GGTGGCCATC   60
Afr1    1 ATGCGCGTCG  GTATTCCGAC  CGAGACCAAA  AACAACG---  ---AATTCCG  GGTGGCCATC   60
Mic1    1 ATGCGCGTCG  GTATTCCGAC  CGAGACCAAA  AACAACG---  ---AATTCCG  GGTGGCCATC   60
-----------------------------------------------------------------------------------

40kD   61 ACCCCGGCCG  GCGTCGCGGA  ACTAACCCGT  CGTGGCCATG  AGGTGCTCAT  CCAGGCAGGT   120
Tub1   61 ACCCCGGCCG  GCGTCGCGGA  ACTAACCCGT  CGTGGCCATG  AGGTGCTCAT  CCAGGCAGGT   120
H37Rv  61 ACCCCGGCCG  GCGTCGCGGA  ACTAACCCGT  CGTGGCCATG  AGGTGCTCAT  CCAGGCAGGT   120
H37Ra  61 ACCCCGGCCG  GCGTCGCGGA  ACTAACCCGT  CGTGGCCATG  AGGTGCTCAT  CCAGGCAGGT   120
BCG4   61 ACCCCGGCCG  GCGTCGCGGA  ACTAACCCGT  CGTGGCCATG  AGGTGCTCAT  CCAGGCAGGT   120
BCG2   61 ACCCCGGCCG  GCGTCGCGGA  ACTAACCCGT  CGTGGCCATG  AGGTGCTCAT  CCAGGCAGGT   120
Bov3   61 ACCCCGGCCG  GCGTCGCGGA  ACTAACCCGT  CGTGGCCATG  AGGTGCTCAT  CCAGGCAGGT   120
Afr1   61 ACCCCGGCCG  GCGTCGCGGA  ACTAACCCGT  CGTGGCCATG  AGGTGCTCAT  CCAGGCAGGT   120
Mic1   61 ACCCCGGCCG  GCGTCGCGGA  ACTAACCCGT  CGTGGCCATG  AGGTGCTCAT  CCAGGCAGGT   120
-----------------------------------------------------------------------------------

40kD  121 GCCGGAGAGG  GCTCGGCTAT  CACCGACGCG  GATTTCAAGG  CGGCAGGCGC  GCAACTGGTC   180
Tub1  121 GCCGGAGAGG  GCTCGGCTAT  CACCGACGCG  GATTTCAAGG  CGGCAGGCGC  GCAACTGGTC   180
H37Rv 121 GCCGGAGAGG  GCTCGGCTAT  CACCGACGCG  GATTTCAAGG  CGGCAGGCGC  GCAACTGGTC   180
H37Ra 121 GCCGGAGAGG  GCTCGGCTAT  CACCGACGCG  GATTTCAAGG  CGGCAGGCGC  GCAACTGGTC   180
BCG4  121 GCCGGAGAGG  GCTCGGCTAT  CACCGACGCG  GATTTCAAGG  CGGCAGGCGC  GCAACTGGTC   180
BCG2  121 GCCGGAGAGG  GCTCGGCTAT  CACCGACGCG  GATTTCAAGG  CGGCAGGCGC  GCAACTGGTC   180
Bov3  121 GCCGGAGAGG  GCTCGGCTAT  CACCGACGCG  GATTTCAAGG  CGGCAGGCGC  GCAACTGGTC   180
Afr1  121 GCCGGAGAGG  GCTCGGCTAT  CACCGACGCG  GATTTCAAGG  CGGCAGGCGC  GCAACTGGTC   180
Mic1  121 GCCGGAGAGG  GCTCGGCTAT  CACCGACGCG  GATTTCAAGG  CGGCAGGCGC  GCAACTGGTC   180
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40kD  181 GGCACCGCCG  ACCAGGTGTG  GGCCGACGCT  GATTTATTGC  TCAAGGTCAA  AGAACCGATA   240
Tub1  181 GGCACCGCCG  ACCAGGTGTG  GGCCGACGCT  GATTTATTGC  TCAAGGTCAA  AGAACCGATA   240
H37Rv 181 GGCACCGCCG  ACCAGGTGTG  GGCCGACGCT  GATTTATTGC  TCAAGGTCAA  AGAACCGATA   240
H37Ra 181 GGCACCGCCG  ACCAGGTGTG  GGCCGACGCT  GATTTATTGC  TCAAGGTCAA  AGAACCGATA   240
BCG4  181 GGCACCGCCG  ACCAGGTGTG  GGCCGACGCT  GATTTATTGC  TCAAGGTCAA  AGAACCGATA   240
BCG2  181 GGCACCGCCG  ACCAGGTGTG  GGCCGACGCT  GATTTATTGC  TCAAGGTCAA  AGAACCGATA   240
Bov3  181 GGCACCGCCG  ACCAGGTGTG  GGCCGACGCT  GATTTATTGC  TCAAGGTCAA  AGAACCGATA   240
Afr1  181 GGCACCGCCG  ACCAGGTGTG  GGCCGACGCT  GATTTATTGC  TCAAGGTCAA  AGAACCGATA   240
Mic1  181 GGCACCGCCG  ACCAGGTGTG  GGCCGACGCT  GATTTATTGC  TCAAGGTCAA  AGAACCGATA   240
-----------------------------------------------------------------------------------
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                                                    *
40kD   241 GCGGCGGAAT ACGGCCGCCT GCGACACGGG CAGATCTTGT TCACGTTCTT GCATTTGGCC  300
Tub1   241 GCGGCGGAAT ACGGCCGCCT GCGACACGGG CAGATCTTGT TCACGTTCTT GCATTTGGCC  300
H37Rv  241 GCGGCGGAAT ACGGCCGCCT GCGACACGGG CAGATCTTGT TCACGTTCTT GCATTTGGCC  300
H37Ra  241 GCGGCGGAAT ACGGCCGCCT GCGACACGGG CAGATCTTGT TCACGTTCTT GCATTTGGCC  300
BCG4   241 GCGGCGGAAT ACGGCCGCCT GCGACACGGG C-GATCTTGT TCACGTTCTT GCATTTGGCC  300
BCG2   241 GCGGCGGAAT ACGGCCGCCT GCGACACGGG C-GATCTTGT TCACGTTCTT GCATTTGGCC  300
Bov3   241 GCGGCGGAAT ACGGCCGCCT GCGACACGGG C-GATCTTGT TCACGTTCTT GCATTTGGCC  300
Afr1   241 GCGGCGGAAT ACGGCCGCCT GCGACACGGG CAGATCTTGT TCACGTTCTT GCATTTGGCC  300
Mic1   241 GCGGCGGAAT ACGGCCGCCT GCGACACGGG CAGATCTTGT TCACGTTCTT GCATTTGGCC  300

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40kD   301 GCGTCACGTG CTTGCACCGA TGCGTTGTTG GATTCCGGCA CCACGTCAAT TGCCTACGAG  360
Tub1   301 GCGTCACGTG CTTGCACCGA TGCGTTGTTG GATTCCGGCA CCACGTCAAT TGCCTACGAG  360
H37Rv  301 GCGTCACGTG CTTGCACCGA TGCGTTGTTG GATTCCGGCA CCACGTCAAT TGCCTACGAG  360
H37Ra  301 GCGTCACGTG CTTGCACCGA TGCGTTGTTG GATTCCGGCA CCACGTCAAT TGCCTACGAG  360
BCG4   301 GCGTCACGTG CTTGCACCGA TGCGTTGTTG GATTCCGGCA CCACGTCAAT TGCCTACGAG  360
BCG2   301 GCGTCACGTG CTTGCACCGA TGCGTTGTTG GATTCCGGCA CCACGTCAAT TGCCTACGAG  360
Bov3   301 GCGTCACGTG CTTGCACCGA TGCGTTGTTG GATTCCGGCA CCACGTCAAT TGCCTACGAG  360
Afr1   301 GCGTCACGTG CTTGCACCGA TGCGTTGTTG GATTCCGGCA CCACGTCAAT TGCCTACGAG  360
Mic1   301 GCGTCACGTG CTTGCACCGA TGCGTTGTTG GATTCCGGCA CCACGTCAAT TGCCTACGAG  360

-------------------------------------------------------------------------------

40kD   361 ACCGTCCAGA CCGCCGACGG CGCACTACCC CTGCTTGCCC CGATGAGCGA AGTCGCCGGT  420
Tub1   361 ACCGTCCAGA CCGCCGACGG CGCACTACCC CTGCTTGCCC CGATGAGCGA AGTCGCCGGT  420
H37Rv  361 ACCGTCCAGA CCGCCGACGG CGCACTACCC CTGCTTGCCC CGATGAGCGA AGTCGCCGGT  420
H37Ra  361 ACCGTCCAGA CCGCCGACGG CGCACTACCC CTGCTTGCCC CGATGAGCGA AGTCGCCGGT  420
BCG4   361 ACCGTCCAGA CCGCCGACGG CGCACTACCC CTGCTTGCCC CGATGAGCGA AGTCGCCGGT  420
BCG2   361 ACCGTCCAGA CCGCCGAAGG CGCACTACCC CTGCTTGCCC CGATGAGCGA AGTCGCCGGT  420
Bov3   361 ACCGTCCAGA CCGCCGAAGG CGCACTACCC CTGCTTGCCC CGATGAGCGA AGTCGCCGGT  420
Afr1   361 ACCGTCCAGA CCGCCGACGG CGCACTACCC CTGCTTGCCC CGATGAGCGA AGTCGCCGGT  420
Mic1   361 ACCGTCCAGA CCGCCGACGG CGCACTACCC CTGCTTGCCC CGATGAGCGA AGTCGCCGGT  420

-------------------------------------------------------------------------------

40kD   421 CGACTCGCCG CCCAGGTTGG CGCTTACCAC CTGATGCGAA CCCAAGGGGG CCGCGGTGTG  480
Tub1   421 CGACTCGCCG CCCAGGTTGG CGCTTACCAC CTGATGCGAA CCCAAGGGGG CCGCGGTGTG  480
H37Rv  421 CGACTCGCCG CCCAGGTTGG CGCTTACCAC CTGATGCGAA CCCAAGGGGG CCGCGGTGTG  480
H37Ra  421 CGACTCGCCG CCCAGGTTGG CGCTTACCAC CTGATGCGAA CCCAAGGGGG CCGCGGTGTG  480
BCG4   421 CGACTCGCCG CCCAGGTTGG CGCTTACCAC CTGATGCGAA CCCAAGGGGG CCGCGGTGTG  480
BCG2   421 CGACTCGCCG CCCAGGTTGG CGCTTACCAC CTGATGCGAA CCCAAGGGGG CCGCGGTGTG  480
Bov3   421 CGACTCGCCG CCCAGGTTGG CGCTTACCAC CTGATGCGAA CCCAAGGGGG CCGCGGTGTG  480
Afr1   421 CGACTCGCCG CCCAGGTTGG CGCTTACCAC CTGATGCGAA CCCAAGGGGG CCGCGGTGTG  480
Mic1   421 CGACTCGCCG CCCAGGTTGG CGCTTACCAC CTGATGCGAA CCCAAGGGGG CCGCGGTGTG  480

-------------------------------------------------------------------------------

40kD   481 CTGATGGGCG GGGTGCCCGG CGTCGAACCG GCCGACGTCG TGGTGATCGG CGCCGGCACC  540
Tub1   481 CTGATGGGCG GGGTGCCCGG CGTCGAACCG GCCGACGTCG TGGTGATCGG CGCCGGCACC  540
H37Rv  481 CTGATGGGCG GGGTGCCCGG CGTCGAACCG GCCGACGTCG TGGTGATCGG CGCCGGCACC  540
H37Ra  481 CTGATGGGCG GGGTGCCCGG CGTCGAACCG GCCGACGTCG TGGTGATCGG CGCCGGCACC  540
BCG4   481 CTGATGGGCG GGGTGCCCGG CGTCGAACCG GCCGACGTCG TGGTGATCGG CGCCGGCACC  540
BCG2   481 CTGATGGGCG GGGTGCCCGG CGTCGAACCG GCCGACGTCG TGGTGATCGG CGCCGGCACC  540
Bov3   481 CTGATGGGCG GGGTGCCCGG CGTCGAACCG GCCGACGTCG TGGTGATCGG CGCCGGCACC  540
Afr1   481 CTGATGGGCG GGGTGCCCGG CGTCGAACCG GCCGACGTCG TGGTGATCGG CGCCGGCACC  540
Mic1   481 CTGATGGGCG GGGTGCCCGG CGTCGAACCG GCCGACGTCG TGGTGATCGG CGCCGGCACC  540

-------------------------------------------------------------------------------
```

```
40kD   541 GCCGGCTACA ACGCAGCCCG CATCGCCAAC GGCATGGGCG CGACCGTTAC GGTTCTAGAC  600
Tub1   541 GCCGGCTACA ACGCAGCCCG CATCGCCAAC GGCATGGGCG CGACCGTTAC GGTTCTAGAC  600
H37Rv  541 GCCGGCTACA ACGCAGCCCG CATCGCCAAC GGCATGGGCG CGACCGTTAC GGTTCTAGAC  600
H37Ra  541 GCCGGCTACA ACGCAGCCCG CATCGCCAAC GGCATGGGCG CGACCGTTAC GGTTCTAGAC  600
BCG4   541 GCCGGCTACA ACGCAGCCCG CATCGCCAAC GGCATGGGCG CGACCGTTAC GGTTCTAGAC  600
BCG2   541 GCCGGCTACA ACGCAGCCCG CATCGCCAAC GGCATGGGCG CGACCGTTAC GGTTCTAGAC  600
Bov3   541 GCCGGCTACA ACGCAGCCCG CATCGCCAAC GGCATGGGCG CGACCGTTAC GGTTCTAGAC  600
Afr1   541 GCCGGCTACA ACGCAGCCCG CATCGCCAAC GGCATGGGCG CGACCGTTAC GGTTCTAGAC  600
Mic1   541 GCCGGCTACA ACGCAGCCCG CATCGCCAAC GGCATGGGCG CGACCGTTAC GGTTCTAGAC  600
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40kD   601 ATCAACATCG ACAAACTTCG GCAACTCGAC GCCGAGTTCT GCGGCCGGAT CCACACTCGC  660
Tub1   601 ATCAACATCG ACAAACTTCG GCAACTCGAC GCCGAGTTCT GCGGCCGGAT CCACACTCGC  660
H37Rv  601 ATCAACATCG ACAAACTTCG GCAACTCGAC GCCGAGTTCT GCGGCCGGAT CCACACTCGC  660
H37Ra  601 ATCAACATCG ACAAACTTCG GCAACTCGAC GCCGAGTTCT GCGGCCGGAT CCACACTCGC  660
BCG4   601 ATCAACATCG ACAAACTTCG GCAACTCGAC GCCGAGTTCT GCGGCCGGAT CCACACTCGC  660
BCG2   601 ATCAACATCG ACAAACTTCG GCAACTCGAC GCCGAGTTCT GCGGCCGGAT CCACACTCGC  660
Bov3   601 ATCAACATCG ACAAACTTCG GCAACTCGAC GCCGAGTTCT GCGGCCGGAT CCACACTCGC  660
Afr1   601 ATCAACATCG ACAAACTTCG GCAACTCGAC GCCGAGTTCT GCGGCCGGAT CCACACTCGC  660
Mic1   601 ATCAACATCG ACAAACTTCG GCAACTCGAC GCCGAGTTCT GCGGCCGGAT CCACACTCGC  660
---------------------------------------------------------------------------
40kD   661 TACTCATCGG CCTACGAGCT CGAGGGTGCC GTCAAACGTG CCGACCTGGT GATTGGGGCC  720
Tub1   661 TACTCATCGG CCTACGAGCT CGAGGGTGCC GTCAAACGTG CCGACCTGGT GATTGGGGCC  720
H37Rv  661 TACTCATCGG CCTACGAGCT CGAGGGTGCC GTCAAACGTG CCGACCTGGT GATTGGGGCC  720
H37Ra  661 TACTCATCGG CCTACGAGCT CGAGGGTGCC GTCAAACGTG CCGACCTGGT GATTGGGGCC  720
BCG4   661 TACTCATCGG CCTACGAGCT CGAGGGTGCC GTCAAACGTG CCGACCTGGT GATTGGGGCC  720
BCG2   661 TACTCATCGG CCTACGAGCT CGAGGGTGCC GTCAAACGTG CCGACCTGGT GATTGGGGCC  720
Bov3   661 TACTCATCGG CCTACGAGCT CGAGGGTGCC GTCAAACGTG CCGACCTGGT GATTGGGGCC  720
Afr1   661 TACTCATCGG CCTACGAGCT CGAGGGTGCC GTCAAACGTG CCGACCTGGT GATTGGGGCC  720
Mic1   661 TACTCATCGG CCTACGAGCT CGAGGGTGCC GTCAAACGTG CCGACCTGGT GATTGGGGCC  720
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40kD   721 GTCCTGGTGC CAGGCGCCAA GGCACCCAAA TTAGTCTCGA ATTCACTTGT CGCGCATATG  780
Tub1   721 GTCCTGGTGC CAGGCGCCAA GGCACCCAAA TTAGTCTCGA ATTCACTTGT CGCGCATATG  780
H37Rv  721 GTCCTGGTGC CAGGCGCCAA GGCACCCAAA TTAGTCTCGA ATTCACTTGT CGCGCATATG  780
H37Ra  721 GTCCTGGTGC CAGGCGCCAA GGCACCCAAA TTAGTCTCGA ATTCACTTGT CGCGCATATG  780
BCG4   721 GTCCTGGTGC CAGGCGCCAA GGCACCCAAA TTAGTCTCGA ATTCACTTGT CGCGCATATG  780
BCG2   721 GTCCTGGTGC CAGGCGCCAA GGCACCCAAA TTAGTCTCGA ATTCACTTGT CGCGCATATG  780
Bov3   721 GTCCTGGTGC CAGGCGCCAA GGCACCCAAA TTAGTCTCGA ATTCACTTGT CGCGCATATG  780
Afr1   721 GTCCTGGTGC CAGGCGCCAA GGCACCCAAA TTAGTCTCGA ATTCACTTGT CGCGCATATG  780
Mic1   721 GTCCTGGTGC CAGGCGCCAA GGCACCCAAA TTAGTCTCGA ATTCACTTGT CGCGCATATG  780
---------------------------------------------------------------------------
40kD   781 AAACCAGGTG CGGTACTGGT GGATATAGCC ATCGACCAGG GCGGCTGTTT CGAAGGCTCA  840
Tub1   781 AAACCAGGTG CGGTACTGGT GGATATAGCC ATCGACCAGG GCGGCTGTTT CGAAGGCTCA  840
H37Rv  781 AAACCAGGTG CGGTACTGGT GGATATAGCC ATCGACCAGG GCGGCTGTTT CGAAGGCTCA  840
H37Ra  781 AAACCAGGTG CGGTACTGGT GGATATAGCC ATCGACCAGG GCGGCTGTTT CGAAGGCTCA  840
BCG4   781 AAACCAGGTG CGGTACTGGT GGATATAGCC ATCGACCAGG GCGGCTGTTT CGAAGGCTCA  840
BCG2   781 AAACCAGGTG CGGTACTGGT GGATATAGCC ATCGACCAGG GCGGCTGTTT CGAAGGCTCA  840
Bov3   781 AAACCAGGTG CGGTACTGGT GGATATAGCC ATCGACCAGG GCGGCTGTTT CGAAGGCTCA  840
Afr1   781 AAACCAGGTG CGGTACTGGT GGATATAGCC ATCGACCAGG GCGGCTGTTT CGAAGGCTCA  840
Mic1   781 AAACCAGGTG CGGTACTGGT GGATATAGCC ATCGACCAGG GCGGCTGTTT CGAAGGCTCA  840
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40kD	841	CGACCGACCA	CCTACGACCA	CCCGACGTTC	GCCGTGCACG	ACACGCTGTT	TTACTGCGTG	900
Tub1	841	CGACCGACCA	CCTACGACCA	CCCGACGTTC	GCCGTGCACG	ACACGCTGTT	TTACTGCGTG	900
H37Rv	841	CGACCGACCA	CCTACGACCA	CCCGACGTTC	GCCGTGCACG	ACACGCTGTT	TTACTGCGTG	900
H37Ra	841	CGACCGACCA	CCTACGACCA	CCCGACGTTC	GCCGTGCACG	ACACGCTGTT	TTACTGCGTG	900
BCG4	841	CGACCGACCA	CCTACGACCA	CCCGACGTTC	GCCGTGCACG	ACACGCTGTT	TTACTGCGTG	900
BCG2	841	CGACCGACCA	CCTACGACCA	CCCGACGTTC	GCCGTGCACG	ACACGCTGTT	TTACTGCGTG	900
Bov3	841	CGACCGACCA	CCTACGACCA	CCCGACGTTC	GCCGTGCACG	ACACGCTGTT	TTACTGCGTG	900
Afr1	841	CGACCGACCA	CCTACGACCA	CCCGACGTTC	GCCGTGCACG	ACACGCTGTT	TTACTGCGTG	900
Mic1	841	CGACCGACCA	CCTACGACCA	CCCGACGTTC	GCCGTGCACG	ACACGCTGTT	TTACTGCGTG	900

40kD	901	GCGAACATGC	CCGCCTCGGT	GCCGAAGACG	TCGACCTACG	CGCTGACCAA	CGCGACGATG	960
Tub1	901	GCGAACATGC	CCGCCTCGGT	GCCGAAGACG	TCGACCTACG	CGCTGACCAA	CGCGACGATG	960
H37Rv	901	GCGAACATGC	CCGCCTCGGT	GCCGAAGACG	TCGACCTACG	CGCTGACCAA	CGCGACGATG	960
H37Ra	90	GCGAACATGC	CCGCCTCGGT	GCCGAAGACG	TCGACCTACG	CGCTGACCAA	CGCGACGATG	960
BCG4	90	GCGAACATGC	CCGCCTCGGT	GCCGAAGACG	TCGACCTACG	CGCTGACCAA	CGCGACGATG	960
BCG2	901	GCGAACATGC	CCGCCTCGGT	GCCGAAGACG	TCGACCTACG	CGCTGACCAA	CGCGACGATG	960
Bov3	901	GCGAACATGC	CCGCCTCGGT	GCCGAAGACG	TCGACCTACG	CGCTGACCAA	CGCGACGATG	960
Afr1	901	GCGAACATGC	CCGCCTCGGT	GCCGAAGACG	TCGACCTACG	CGCTGACCAA	CGCGACGATG	960
Mic1	901	GCGAACATGC	CCGCCTCGGT	GCCGAAGACG	TCGACCTACG	CGCTGACCAA	CGCGACGATG	960

40kD	961	CCGTATGTGC	TCGAGCTTGC	CGACCATGGC	TGGCGGGCGG	CGTGCCGGTC	GAATCCGGCA	1020
Tub1	961	CCGTATGTGC	TCGAGCTTGC	CGACCATGGC	TGGCGGGCGG	CGTGCCGGTC	GAATCCGGCA	1020
H37Rv	961	CCGTATGTGC	TCGAGCTTGC	CGACCATGGC	TGGCGGGCGG	CGTGCCGGTC	GAATCCGGCA	1020
H37Ra	961	CCGTATGTGC	TCGAGCTTGC	CGACCATGGC	TGGCGGGCGG	CGTGCCGGTC	GAATCCGGCA	1020
BCG4	961	CCGTATGTGC	TCGAGCTTGC	CGACCATGGC	TGGCGGGCGG	CGTGCCGGTC	GAATCCGGCA	1020
BCG2	961	CCGTATGTGC	TCGAGCTTGC	CGACCATGGC	TGGCGGGCGG	CGTGCCGGTC	GAATCCGGCA	1020
Bov3	961	CCGTATGTGC	TCGAGCTTGC	CGACCATGGC	TGGCGGGCGG	CGTGCCGGTC	GAATCCGGCA	1020
Afr1	961	CCGTATGTGC	TCGAGCTTGC	CGACCATGGC	TGGCGGGCGG	CGTGCCGGTC	GAATCCGGCA	1020
Mic1	961	CCGTATGTGC	TCGAGCTTGC	CGACCATGGC	TGGCGGGCGG	CGTGCCGGTC	GAATCCGGCA	1020

40kD	1021	CTAGCCAAAG	GTCTTTCGAC	GCACGAAGGG	GCGTTACTGT	CCGAACGGGT	GGCCACCGAC	1080
Tub1	1021	CTAGCCAAAG	GTCTTTCGAC	GCACGAAGGG	GCGTTACTGT	CCGAACGGGT	GGCCACCGAC	1080
H37Rv	1021	CTAGCCAAAG	GTCTTTCGAC	GCACGAAGGG	GCGTTACTGT	CCGAACGGGT	GGCCACCGAC	1080
H37Ra	1021	CTAGCCAAAG	GTCTTTCGAC	GCACGAAGGG	GCGTTACTGT	CCGAACGGGT	GGCCACCGAC	1080
BCG4	1021	CTAGCCAAAG	GTCTTTCGAC	GCACGAAGGG	GCGTTACTGT	CCGAACGGGT	GGCCACCGAC	1080
BCG2	1021	CTAGCCAAAG	GTCTTTCGAC	GCACGAAGGG	GCGTTACTGT	CCGAACGGGT	GGCCACCGAC	1080
Bov3	1021	CTAGCCAAAG	GTCTTTCGAC	GCACGAAGGG	GCGTTACTGT	CCGAACGGGT	GGCCACCGAC	1080
Afr1	1021	CTAGCCAAAG	GTCTTTCGAC	GCACGAAGGG	GCGTTACTGT	CCGAACGGGT	GGCCACCGAC	1080
Mic1	1021	CTAGCCAAAG	GTCTTTCGAC	GCACGAAGGG	GCGTTACTGT	CCGAACGGGT	GGCCACCGAC	1080

Stop

40kD	1081	CTGGGGGTGC	CGTTCACCGA	GCCCGCCAGC	GTGCTGGCCT	GACTCTCGGC	CGCTCGTTAC	1140
Tub1	1081	CTGGGGGTGC	CGTTCACCGA	GCCCGCCAGC	GTGCTGGCCT	GACTCTCGGC	CGCTCGTTAC	1140
H37Rv	1081	CTGGGGGTGC	CGTTCACCGA	GCCCGCCAGC	GTGCTGGCCT	GACTCTCGGC	CGCTCGTTAC	1140
H37Ra	1081	CTGGGGGTGC	CGTTCACCGA	GCCCGCCAGC	GTGCTGGCCT	GACTCTCGGC	CGCTCGTTAC	1140
BCG4	1081	CTGGGGGTGC	CGTTCACCGA	GCCCGCCAGC	GTGCTGGCCT	GACTCTCGGC	CGCTCGTTAC	1140
BCG2	1081	CTGGGGGTGC	CGTTCACCGA	GCCCGCCAGC	GTGCTGGCCT	GACTCTCGGC	CGCTCGTTAC	1140
Bov3	1081	CTGGGGGTGC	CGTTCACCGA	GCCCGCCAGC	GTGCTGGCCT	GACTCTCGGC	CGCTCGTTAC	1140
Afr1	1081	CTGGGGGTGC	CGTTCACCGA	GCCCGCCAGC	GTGCTGGCCT	GACTCTCGGC	CGCTCGTTAC	1140
Mic1	1081	CTGGGGGTGC	CGTTCACCGA	GCCCGCCAGC	GTGCTGGCCT	GACTCTCGGC	CGCTCGTTAC	1140

40kD	1141	GCCGAGCACA	CGTCGGGAGT	AAGGGAAGCG	ATGATGTCGG	CCGCG	1185
Tub1	1141	GCCGAGCACA	CNTCGGGAGT	AANGGAAGCG	ATGATGTCGN	C	1185
H37Rv	1141	GCCGAGCACA	CGTCGGGAGT	AAGGGAAGCG	ATGATGTCGG	CCG	1185
H37Ra	1141	GCCGAGCACA	CGTCGGGAGT	AAGGGAAGCG	ATGA		1185
BCG4	1141	GCCGANCACA	CGTCGGGAGT	AAGGGAAGCG	ATGATGTCGG	CC	1185
BCG2	1141	GCCGAGCACA	CGTCNGGAGT	AAGGGAAGCG	ATGATG		1185
Bov3	1141	GCCGAGCACA	CGTCGGGAGT	AAGGGAAGCG	ATGATGTCGG	CC	1185
Afr1	1141	GCCGAGCNCA	CGTCG				1185
Mic1	1141	GCCGAGCACA	CGTCGGGAGT	AAGGGAAGCG	ATGATGTCGG	CC	1185

Fig. 3.19

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Flohé, Leopold
Singh, Mahavir
Hutter, Bernd
Kolk, Arend

(ii) TITLE OF INVENTION: Test-Kit For Tuberculosis Diagnosis etc.

(iii) NUMBER OF SEQUENCES: 29

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Marshall, O'Toole, Gerstein, Murray & Borun
(B) STREET: 233 South Wacker Drive/6300 Sears Tower
(C) CITY: Chicago
(D) STATE: Illinois
(E) COUNTRY: United States of America
(F) ZIP: 60606

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: WO PCT/EP98/00483
(B) FILING DATE: 29-JAN-1998

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: EP 97101338.8
(B) FILING DATE: 29-JAN-1997

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Zeller, James P.
(B) REGISTRATION NUMBER: 28,491
(C) REFERENCE/DOCKET NUMBER: 29473/35834

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: (312) 474-6300
(B) TELEFAX: (312) 474-0448

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1260 base pairs

(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

GAATTCCCAT CAGCAATCTT GCAGATTAAT CGAACTTTCT TCACACTGAA GCGTACAGTA 60

TCGAGAGGGG TAATCATGCG CGTCGGTATT CCGACCGAGA CCAAAAACAA CGAATTCCAA 120

TTCCGGGTGG CCATCACCCC GGCCGGCGTC GCGGAACTAA CCCGTCGTGG CCATGAGGTG 180

CTCATCCAGG CAGGTGCCGG AGAGGGCTCG GCTATCACCG ACGCGGATTT CAAGGCGGCA 240

GGCGCGCAAC TGGTCGGCAC CGCCGACCAG GTGTGGGCCG ACGCTGATTT ATTGCTCAAG 300

GTCAAAGAAC CGATAGCGGC GGAATACGGC CGCCTGCGAC ACGGGCAGAT CTTGTTCACG 360

TTCTTGCATT TGGCCGCGTC ACGTGCTTGC ACCGATGCGT TGTTGGATTC CGGCACCACG 420

TCAATTGCCT ACGAGACCGT CCAGACCGCC GACGGCGCAC TACCCCTGCT TGCCCCGATG 480

AGCGAAGTCG CCGGTCGACT CGCCGCCCAG GTTGGCGCTT ACCACCTGAT GCGAACCCAA 540

GGGGGCCGCG GTGTGCTGAT GGGCGGGGTG CCCGGCGTCG AACCGGCCGA CGTCGTGGTG 600

ATCGGCGCCG GCACCGCCGG CTACAACGCA GCCCGCATCG CCAACGGCAT GGGCGCGACC 660

GTTACGGTTC TAGACATCAA CATCGACAAA CTTCGGCAAC TCGACGCCGA GTTCTGCGGC 720

CGGATCCACA CTCGCTACTC ATCGGCCTAC GAGCTCGAGG GTGCCGTCAA ACGTGCCGAC 780

CTGGTGATTG GGGCCGTCCT GGTGCCAGGC GCCAAGGCAC CCAAATTAGT CTCGAATTCA 840

CTTGTCGCGC ATATGAAACC AGGTGCGGTA CTGGTGGATA TAGCCATCGA CCAGGGCGGC 900

TGTTTCGAAG GCTCACGACC GACCACCTAC GACCACCCGA CGTTCGCCGT GCACGACACG 960

CTGTTTTACT GCGTGGCGAA CATGCCCGCC TCGGTGCCGA AGACGTCGAC CTACGCGCTG 1020

ACCAACGCGA CGATGCCGTA TGTGCTCGAG CTTGCCGACC ATGGCTGGCG GGCGGCGTGC 1080

CGGTCGAATC CGGCACTAGC CAAAGGTCTT TCGACGCACG AAGGGGCGTT ACTGTCCGAA 1140

CGGGTGGCCA CCGACCTGGG GGTGCCGTTC ACCGAGCCCG CCAGCGTGCT GGCCTGACTC 1200

TCGGCCGCTC GTTACGCCGA GCACACGTCG GGAGTAAGGG AAGCGATGAT GTCGGCCGCG 1260

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1245 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

ATCTTGCAGA TTAATCGAAC TTTCTTCACA CTGAAGCGTA CAGTATCGAG AGGGGTAATC 60

ATGCGCGTCG GTATTCCGAC CGAGACCAAA AACAACGAAT TCCAATTCCG GGTGGCCATC 120

ACCCCGGCCG GCGTCGCGGA ACTAACCCGT CGTGGCCATG AGGTGCTCAT CCAGGCAGGT 180

GCCGGAGAGG GCTCGGCTAT CACCGACGCG GATTTCAAGG CGGCAGGCGC GCAACTGGTC 240

GGCACCGCCG ACCAGGTGTG GGCCGACGCT GATTTATTGC TCAAGGTCAA AGAACCGATA 300

GCGGCGGAAT ACGGCCGCCT GCGACACGGG CAGATCTTGT TCACGTTCTT GCATTTGGCC 360

GCGTCACGTG CTTGCACCGA TGCGTTGTTG GATTCCGGCA CCACGTCAAT TGCCTACGAG 420

ACCGTCCAGA CCGCCGACGG CGCACTACCC CTGCTTGCCC CGATGAGCGA AGTCGCCGGT 480

CGACTCGCCG CCCAGGTTGG CGCTTACCAC CTGATGCGAA CCCAAGGGGG CCGCGGTGTG 540

CTGATGGGCG GGGTGCCCGG CGTCGAACCG GCCGACGTCG TGGTGATCGG CGCCGGCACC 600

GCCGGCTACA ACGCAGCCCG CATCGCCAAC GGCATGGGCG CGACCGTTAC GGTTCTAGAC 660

ATCAACATCG ACAAACTTCG GCAACTCGAC GCCGAGTTCT GCGGCCGGAT CCACACTCGC 720

TACTCATCGG CCTACGAGCT CGAGGGTGCC GTCAAACGTG CCGACCTGGT GATTGGGGCC 780

GTCCTGGTGC CAGGCGCCAA GGCACCCAAA TTAGTCTCGA ATTCACTTGT CGCGCATATG 840

AAACCAGGTG CGGTACTGGT GGATATAGCC ATCGACCAGG GCGGCTGTTT CGAAGGCTCA 900

CGACCGACCA CCTACGACCA CCCGACGTTC GCCGTGCACG ACACGCTGTT TTACTGCGTG 960

GCGAACATGC CCGCCTCGGT GCCGAAGACG TCGACCTACG CGCTGACCAA CGCGACGATG 1020

CCGTATGTGC TCGAGCTTGC CGACCATGGC TGGCGGGCGG CGTGCCGGTC GAATCCGGCA 1080

CTAGCCAAAG GTCTTTCGAC GCACGAAGGG GCGTTACTGT CCGAACGGGT GGCCACCGAC 1140

CTGGGGGTGC CGTTCACCGA GCCCGCCAGC GTGCTGGCCT GACTCTCGGC CGCTCGTTAC 1200

GCCGAGCACA CGTCGGGAGT AAGGGAAGCG ATGATGTCGG CCGCG 1245

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1235 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

ATCTTGCAGA TTAATCGAAC TTTCTTCACA CTGAAGCGTA CAGTATCGAG AGGGGTAATC 60

ATGCGCGTCG GTATTCCGAC CGAGACCAAA AACAACGAAT TCCGGGTGGC CATCACCCCG 120

GCCGGCGTCG CGGAACTAAC CCGTCGTGGC CATGAGGTGC TCATCCAGGC AGGTGCCGGA 180

GAGGGCTCGG CTATCACCGA CGCGGATTTC AAGGCGGCAG GCGCGCAACT GGTCGGCACC 240

GCCGACCAGG TGTGGGCCGA CGCTGATTTA TTGCTCAAGG TCAAAGAACC GATAGCGGCG 300

GAATACGGCC GCCTGCGACA CGGGCAGATC TTGTTCACGT TCTTGCATTT GGCCGCGTCA 360

CGTGCTTGCA CCGATGCGTT GTTGGATTCC GGCACCACGT CAATTGCCTA CGAGACCGTC 420

CAGACCGCCG ACGGCGCACT ACCCCTGCTT GCCCCGATGA GCGAAGTCGC CGGTCGACTC 480

GCCGCCCAGG TTGGCGCTTA CCACCTGATG CGAACCCAAG GGGGCCGCGG TGTGCTGATG 540

GGCGGGGTGC CCGGCGTCGA ACCGGCCGAC GTCGTGGTGA TCGGCGCCGG CACCGCCGGC 600

TACAACGCAG CCCGCATCGC CAACGGCATG GGCGCGACCG TTACGGTTCT AGACATCAAC 660

ATCGACAAAC TTCGGCAACT CGACGCCGAG TTCTGCGGCC GGATCCACAC TCGCTACTCA 720

TCGGCCTACG AGCTCGAGGG TGCCGTCAAA CGTGCCGACC TGGTGATTGG GGCCGTCCTG 780

GTGCCAGGCG CCAAGGCACC CAAATTAGTC TCGAATTCAC TTGTCGCGCA TATGAAACCA 840

GGTGCGGTAC TGGTGGATAT AGCCATCGAC CAGGGCGGCT GTTTCGAAGG CTCACGACCG 900

ACCACCTACG ACCACCCGAC GTTCGCCGTG CACGACACGC TGTTTTACTG CGTGGCGAAC 960

ATGCCCGCCT CGGTGCCGAA GACGTCGACC TACGCGCTGA CCAACGCGAC GATGCCGTAT 1020

GTGCTCGAGC TTGCCGACCA TGGCTGGCGG GCGGCGTGCC GGTCGAATCC GGCACTAGCC 1080

AAAGGTCTTT CGACGCACGA AGGGGCGTTA CTGTCCGAAC GGGTGGCCAC CGACCTGGGG 1140

GTGCCGTTCA CCGAGCCCGC CAGCGTGCTG GCCTGACTCT CGGCCGCTCG TTACGCCGAG 1200

CACACNTCGG GAGTAANGGA AGCGATGATG TCGNC 1235

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1237 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

ATCTTGCAGA TTAATCGAAC TTTCTTCATA CTGAAGCGTA CAGTATCGAG AGGGGTAATC 60

ATGCGCGTCG GTATTCCGAC CGAGACCAAA AACAACGAAT TCCGGGTGGC CATCACCCCG 120

GCCGGCGTCG CGGAACTAAC CCGTCGTGGC CATGAGGTGC TCATCCAGGC AGGTGCCGGA 180

GAGGGCTCGG CTATCACCGA CGCGGATTTC AAGGCGGCAG GCGCGCAACT GGTCGGCACC 240

GCCGACCAGG TGTGGGCCGA CGCTGATTTA TTGCTCAAGG TCAAAGAACC GATAGCGGCG 300

GAATACGGCC GCCTGCGACA CGGGCAGATC TTGTTCACGT TCTTGCATTT GGCCGCGTCA 360

CGTGCTTGCA CCGATGCGTT GTTGGATTCC GGCACCACGT CAATTGCCTA CGAGACCGTC 420

CAGACCGCCG ACGGCGCACT ACCCCTGCTT GCCCCGATGA GCGAAGTCGC CGGTCGACTC 480

GCCGCCCAGG TTGGCGCTTA CCACCTGATG CGAACCCAAG GGGGCCGCGG TGTGCTGATG 540

GGCGGGGTGC CCGGCGTCGA ACCGGCCGAC GTCGTGGTGA TCGGCGCCGG CACCGCCGGC 600

TACAACGCAG CCCGCATCGC CAACGGCATG GGCGCGACCG TTACGGTTCT AGACATCAAC 660

ATCGACAAAC TTCGGCAACT CGACGCCGAG TTCTGCGGCC GGATCCACAC TCGCTACTCA 720

TCGGCCTACG AGCTCGAGGG TGCCGTCAAA CGTGCCGACC TGGTGATTGG GGCCGTCCTG 780

GTGCCAGGCG CCAAGGCACC CAAATTAGTC TCGAATTCAC TTGTCGCGCA TATGAAACCA 840

GGTGCGGTAC TGGTGGATAT AGCCATCGAC CAGGGCGGCT GTTTCGAAGG CTCACGACCG 900

ACCACCTACG ACCACCCGAC GTTCGCCGTG CACGACACGC TGTTTTACTG CGTGGCGAAC 960

ATGCCCGCCT CGGTGCCGAA GACGTCGACC TACGCGCTGA CCAACGCGAC GATGCCGTAT 1020

GTGCTCGAGC TTGCCGACCA TGGCTGGCGG GCGGCGTGCC GGTCGAATCC GGCACTAGCC 1080

AAAGGTCTTT CGACGCACGA AGGGGCGTTA CTGTCCGAAC GGGTGGCCAC CGACCTGGGG 1140

GTGCCGTTCA CCGAGCCCGC CAGCGTGCTG GCCTGACTCT CGGCCGCTCG TTACGCCGAG 1200

CACACGTCGG GAGTAAGGGA AGCGATGATG TCGGCCG 1237

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1228 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

ATCTTGCAGA TTAATCGAAC TTTCTTCATA CTGAAGCGTA CAGTATCGAG AGGGGTAATC 60

ATGCGCGTCG GTATTCCGAC CGAGACCAAA AACAACGAAT TCCGGGTGGC CATCACCCCG 120

GCCGGCGTCG CGGAACTAAC CCGTCGTGGC CATGAGGTGC TCATCCAGGC AGGTGCCGGA 180

GAGGGCTCGG CTATCACCGA CGCGGATTTC AAGGCGGCAG GCGCGCAACT GGTCGGCACC 240

GCCGACCAGG TGTGGGCCGA CGCTGATTTA TTGCTCAAGG TCAAAGAACC GATAGCGGCG 300

GAATACGGCC GCCTGCGACA CGGGCAGATC TTGTTCACGT TCTTGCATTT GGCCGCGTCA 360

CGTGCTTGCA CCGATGCGTT GTTGGATTCC GGCACCACGT CAATTGCCTA CGAGACCGTC 420

CAGACCGCCG ACGGCGCACT ACCCCTGCTT GCCCCGATGA GCGAAGTCGC CGGTCGACTC 480

GCCGCCCAGG TTGGCGCTTA CCACCTGATG CGAACCCAAG GGGGCCGCGG TGTGCTGATG 540

GGCGGGGTGC CCGGCGTCGA ACCGGCCGAC GTCGTGGTGA TCGGCGCCGG CACCGCCGGC 600

TACAACGCAG CCCGCATCGC CAACGGCATG GGCGCGACCG TTACGGTTCT AGACATCAAC 660

ATCGACAAAC TTCGGCAACT CGACGCCGAG TTCTGCGGCC GGATCCACAC TCGCTACTCA 720

TCGGCCTACG AGCTCGAGGG TGCCGTCAAA CGTGCCGACC TGGTGATTGG GGCCGTCCTG 780

GTGCCAGGCG CCAAGGCACC CAAATTAGTC TCGAATTCAC TTGTCGCGCA TATGAAACCA 840

GGTGCGGTAC TGGTGGATAT AGCCATCGAC CAGGGCGGCT GTTTCGAAGG CTCACGACCG 900

ACCACCTACG ACCACCCGAC GTTCGCCGTG CACGACACGC TGTTTTACTG CGTGGCGAAC 960

ATGCCCGCCT CGGTGCCGAA GACGTCGACC TACGCGCTGA CCAACGCGAC GATGCCGTAT 1020

GTGCTCGAGC TTGCCGACCA TGGCTGGCGG GCGGCGTGCC GGTCGAATCC GGCACTAGCC 1080

AAAGGTCTTT CGACGCACGA AGGGGCGTTA CTGTCCGAAC GGGTGGCCAC CGACCTGGGG 1140

GTGCCGTTCA CCGAGCCCGC CAGCGTGCTG GCCTGACTCT CGGCCGCTCG TTACGCCGAG 1200

CACACGTCGG GAGTAAGGGA AGCGATGA 1228

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1235 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

ATCTTGCAGA TTAATCGAAC TTTCTTCACA CTGAAGCGTA CAGTATCGAG AGGGGTAATC 60

ATGCGCGTCG GTATTCCGAC CGAGACCAAA AACAACGAAT TCCGGGTGGC CATCACCCCG 120

GCCGGCGTCG CGGAACTAAC CCGTCGTGGC CATGAGGTGC TCATCCAGGC AGGTGCCGGA 180

GAGGGCTCGG CTATCACCGA CGCGGATTTC AAGGCGGCAG GCGCGCAACT GGTCGGCACC 240

GCCGACCAGG TGTGGGCCGA CGCTGATTTA TTGCTCAAGG TCAAAGAACC GATAGCGGCG 300

GAATACGGCC GCCTGCGACA CGGGCGATCT TGTTCACGTT CTTGCATTTG GCCGCGTCAC 360

GTGCTTGCAC CGATGCGTTG TTGGATTCCG GCACCACGTC AATTGCCTAC GAGACCGTCC 420

AGACCGCCGA CGGCGCACTA CCCCTGCTTG CCCCGATGAG CGAAGTCGCC GGTCGACTCG 480

CCGCCCAGGT TGGCGCTTAC CACCTGATGC GAACCCAAGG GGGCCGCGGT GTGCTGATGG 540

GCGGGGTGCC CGGCGTCGAA CCGGCCGACG TCGTGGTGAT CGGCGCCGGC ACCGCCGGCT 600

ACAACGCAGC CCGCATCGCC AACGGCATGG GCGCGACCGT TACGGTTCTA GACATCAACA 660

TCGACAAACT TCGGCAACTC GACGCCGAGT TCTGCGGCCG GATCCACACT CGCTACTCAT 720

CGGCCTACGA GCTCGAGGGT GCCGTCAAAC GTGCCGACCT GGTGATTGGG GCCGTCCTGG 780

TGCCAGGCGC CAAGGCACCC AAATTAGTCT CGAATTCACT TGTCGCGCAT ATGAAACCAG 840

GTGCGGTACT GGTGGATATA GCCATCGACC AGGGCGGCTG TTTCGAAGGC TCACGACCGA 900

CCACCTACGA CCACCCGACG TTCGCCGTGC ACGACACGCT GTTTTACTGC GTGGCGAACA 960

TGCCCGCCTC GGTGCCGAAG ACGTCGACCT ACGCGCTGAC CAACGCGACG ATGCCGTATG 1020

TGCTCGAGCT TGCCGACCAT GGCTGGCGGG CGGCGTGCCG GTCGAATCCG GCACTAGCCA 1080

AAGGTCTTTC GACGCACGAA GGGGCGTTAC TGTCCGAACG GGTGGCCACC GACCTGGGGG 1140

TGCCGTTCAC CGAGCCCGCC AGCGTGCTGG CCTGACTCTC GGCCGCTCGT TACGCCGANC 1200

ACACGTCGGG AGTAAGGGAA GCGATGATGT CGGCC 1235

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1229 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

ATCTTGCAGA TTAATCGAAC TTTCTTCACA CTGAAGCGTA CAGTATCGAG AGGGGTAATC 60

ATGCGCGTCG GTATTCCGAC CGAGACCAAA AACAACGAAT TCCGGGTGGC CATCACCCCG 120

GCCGGCGTCG CGGAACTAAC CCGTCGTGGC CATGAGGTGC TCATCCAGGC AGGTGCCGGA 180

GAGGGCTCGG CTATCACCGA CGCGGATTTC AAGGCGGCAG GCGCGCAACT GGTCGGCACC 240

GCCGACCAGG TGTGGGCCGA CGCTGATTTA TTGCTCAAGG TCAAAGAACC GATAGCGGCG 300

GAATACGGCC GCCTGCGACA CGGGCGATCT TGTTCACGTT CTTGCATTTG GCCGCGTCAC 360

GTGCTTGCAC CGATGCGTTG TTGGATTCCG GCACCACGTC AATTGCCTAC GAGACCGTCC 420

AGACCGCCGA AGGCGCACTA CCCCTGCTTG CCCCGATGAG CGAAGTCGCC GGTCGACTCG 480

CCGCCCAGGT TGGCGCTTAC CACCTGATGC GAACCCAAGG GGGCCGCGGT GTGCTGATGG 540

GCGGGGTGCC CGGCGTCGAA CCGGCCGACG TCGTGGTGAT CGGCGCCGGC ACCGCCGGCT 600

ACAACGCAGC CCGCATCGCC AACGGCATGG GCGCGACCGT TACGGTTCTA GACATCAACA 660

TCGACAAACT TCGGCAACTC GACGCCGAGT TCTGCGGCCG GATCCACACT CGCTACTCAT 720

CGGCCTACGA GCTCGAGGGT GCCGTCAAAC GTGCCGACCT GGTGATTGGG GCCGTCCTGG 780

TGCCAGGCGC CAAGGCACCC AAATTAGTCT CGAATTCACT TGTCGCGCAT ATGAAACCAG 840

GTGCGGTACT GGTGGATATA GCCATCGACC AGGGCGGCTG TTTCGAAGGC TCACGACCGA 900

CCACCTACGA CCACCCGACG TTCGCCGTGC ACGACACGCT GTTTTACTGC GTGGCGAACA 960

TGCCCGCCTC GGTGCCGAAG ACGTCGACCT ACGCGCTGAC CAACGCGACG ATGCCGTATG 1020

TGCTCGAGCT TGCCGACCAT GGCTGGCGGG CGGCGTGCCG GTCGAATCCG GCACTAGCCA 1080

AAGGTCTTTC GACGCACGAA GGGGCGTTAC TGTCCGAACG GGTGGCCACC GACCTGGGGG 1140

TGCCGTTCAC CGAGCCCGCC AGCGTGCTGG CCTGACTCTC GGCCGCTCGT TACGCCGAGC 1200

ACACGTCNGG AGTAAGGGAA GCGATGATG 1229

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1235 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

```
ATCTTGCAGA TTAATCGAAC TTTCTTCACA CTGAAGCGTA CAGTATCGAG AGGGGTAATC      60
ATGCGCGTCG GTATTCCGAC CGAGACCAAA AACAACGAAT TCCGGGTGGC CATCACCCCG     120
GCCGGCGTCG CGGAACTAAC CCGTCGTGGC CATGAGGTGC TCATCCAGGC AGGTGCCGGA     180
GAGGGCTCGG CTATCACCGA CGCGGATTTC AAGGCGGCAG GCGCGCAACT GGTCGGCACC     240
GCCGACCAGG TGTGGGCCGA CGCTGATTTA TTGCTCAAGG TCAAAGAACC GATAGCGGCG     300
GAATACGGCC GCCTGCGACA CGGGCGATCT TGTTCACGTT CTTGCATTTG GCCGCGTCAC     360
GTGCTTGCAC CGATGCGTTG TTGGATTCCG GCACCACGTC AATTGCCTAC GAGACCGTCC     420
AGACCGCCGA AGGCGCACTA CCCCTGCTTG CCCCGATGAG CGAAGTCGCC GGTCGACTCG     480
CCGCCCAGGT TGGCGCTTAC CACCTGATGC GAACCCAAGG GGGCCGCGGT GTGCTGATGG     540
GCGGGGTGCC CGGCGTCGAA CCGGCCGACG TCGTGGTGAT CGGCGCCGGC ACCGCCGGCT     600
ACAACGCAGC CCGCATCGCC AACGGCATGG GCGCGACCGT TACGGTTCTA GACATCAACA     660
TCGACAAACT TCGGCAACTC GACGCCGAGT TCTGCGGCCG GATCCACACT CGCTACTCAT     720
CGGCCTACGA GCTCGAGGGT GCCGTCAAAC GTGCCGACCT GGTGATTGGG GCCGTCCTGG     780
TGCCAGGCGC CAAGGCACCC AAATTAGTCT CGAATTCACT TGTCGCGCAT ATGAAACCAG     840
GTGCGGTACT GGTGGATATA GCCATCGACC AGGGCGGCTG TTTCGAAGGC TCACGACCGA     900
CCACCTACGA CCACCCGACG TTCGCCGTGC ACGACACGCT GTTTTACTGC GTGGCGAACA     960
TGCCCGCCTC GGTGCCGAAG ACGTCGACCT ACGCGCTGAC CAACGCGACG ATGCCGTATG    1020
TGCTCGAGCT TGCCGACCAT GGCTGGCGGG CGGCGTGCCG GTCGAATCCG GCACTAGCCA    1080
AAGGTCTTTC GACGCACGAA GGGGCGTTAC TGTCCGAACG GGTGGCCACC GACCTGGGGG    1140
TGCCGTTCAC CGAGCCCGCC AGCGTGCTGG CCTGACTCTC GGCCGCTCGT TACGCCGAGC    1200
ACACGTCGGG AGTAAGGGAA GCGATGATGT CGGCC                               1235
```

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1209 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

ATCTTGCAGA TTAATCGAAC TTTCTTCACA CTGAAGCGTA CAGTATCGAG AGGGGTAATC 60

ATGCGCGTCG GTATTCCGAC CGAGACCAAA AACAACGAAT TCCGGGTGGC CATCACCCCG 120

GCCGGCGTCG CGGAACTAAC CCGTCGTGGC CATGAGGTGC TCATCCAGGC AGGTGCCGGA 180

GAGGGCTCGG CTATCACCGA CGCGGATTTC AAGGCGGCAG GCGCGCAACT GGTCGGCACC 240

GCCGACCAGG TGTGGGCCGA CGCTGATTTA TTGCTCAAGG TCAAAGAACC GATAGCGGCG 300

GAATACGGCC GCCTGCGACA CGGGCAGATC TTGTTCACGT TCTTGCATTT GGCCGCGTCA 360

CGTGCTTGCA CCGATGCGTT GTTGGATTCC GGCACCACGT CAATTGCCTA CGAGACCGTC 420

CAGACCGCCG ACGGCGCACT ACCCCTGCTT GCCCCGATGA GCGAAGTCGC CGGTCGACTC 480

GCCGCCCAGG TTGGCGCTTA CCACCTGATG CGAACCCAAG GGGGCCGCGG TGTGCTGATG 540

GGCGGGGTGC CCGGCGTCGA ACCGGCCGAC GTCGTGGTGA TCGGCGCCGG CACCGCCGGC 600

TACAACGCAG CCCGCATCGC CAACGGCATG GGCGCGACCG TTACGGTTCT AGACATCAAC 660

ATCGACAAAC TTCGGCAACT CGACGCCGAG TTCTGCGGCC GGATCCACAC TCGCTACTCA 720

TCGGCCTACG AGCTCGAGGG TGCCGTCAAA CGTGCCGACC TGGTGATTGG GGCCGTCCTG 780

GTGCCAGGCG CCAAGGCACC CAAATTAGTC TCGAATTCAC TTGTCGCGCA TATGAAACCA 840

GGTGCGGTAC TGGTGGATAT AGCCATCGAC CAGGGCGGCT GTTTCGAAGG CTCACGACCG 900

ACCACCTACG ACCACCCGAC GTTCGCCGTG CACGACACGC TGTTTTACTG CGTGGCGAAC 960

ATGCCCGCCT CGGTGCCGAA GACGTCGACC TACGCGCTGA CCAACGCGAC GATGCCGTAT 1020

GTGCTCGAGC TTGCCGACCA TGGCTGGCGG GCGGCGTGCC GGTCGAATCC GGCACTAGCC 1080

AAAGGTCTTT CGACGCACGA AGGGGCGTTA CTGTCCGAAC GGGTGGCCAC CGACCTGGGG 1140

GTGCCGTTCA CCGAGCCCGC CAGCGTGCTG GCCTGACTCT CGGCCGCTCG TTACGCCGAG 1200

CNCACGTCG 1209

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1236 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

ATCTTGCAGA TTAATCGAAC TTTCTTCACA CTGAAGCGTA CAGTATCGAG AGGGGTAATC 60

ATGCGCGTCG GTATTCCGAC CGAGACCAAA AACAACGAAT TCCGGGTGGC CATCACCCCG 120

GCCGGCGTCG CGGAACTAAC CCGTCGTGGC CATGAGGTGC TCATCCAGGC AGGTGCCGGA 180

GAGGGCTCGG CTATCACCGA CGCGGATTTC AAGGCGGCAG GCGCGCAACT GGTCGGCACC 240

GCCGACCAGG TGTGGGCCGA CGCTGATTTA TTGCTCAAGG TCAAAGAACC GATAGCGGCG 300

GAATACGGCC GCCTGCGACA CGGGCAGATC TTGTTCACGT TCTTGCATTT GGCCGCGTCA 360

CGTGCTTGCA CCGATGCGTT GTTGGATTCC GGCACCACGT CAATTGCCTA CGAGACCGTC 420

CAGACCGCCG ACGGCGCACT ACCCCTGCTT GCCCCGATGA GCGAAGTCGC CGGTCGACTC 480

GCCGCCCAGG TTGGCGCTTA CCACCTGATG CGAACCCAAG GGGGCCGCGG TGTGCTGATG 540

GGCGGGGTGC CCGGCGTCGA ACCGGCCGAC GTCGTGGTGA TCGGCGCCGG CACCGCCGGC 600

TACAACGCAG CCCGCATCGC CAACGGCATG GGCGCGACCG TTACGGTTCT AGACATCAAC 660

ATCGACAAAC TTCGGCAACT CGACGCCGAG TTCTGCGGCC GGATCCACAC TCGCTACTCA 720

TCGGCCTACG AGCTCGAGGG TGCCGTCAAA CGTGCCGACC TGGTGATTGG GGCCGTCCTG 780

GTGCCAGGCG CCAAGGCACC CAAATTAGTC TCGAATTCAC TTGTCGCGCA TATGAAACCA 840

GGTGCGGTAC TGGTGGATAT AGCCATCGAC CAGGGCGGCT GTTTCGAAGG CTCACGACCG 900

ACCACCTACG ACCACCCGAC GTTCGCCGTG CACGACACGC TGTTTTACTG CGTGGCGAAC 960

ATGCCCGCCT CGGTGCCGAA GACGTCGACC TACGCGCTGA CCAACGCGAC GATGCCGTAT 1020

GTGCTCGAGC TTGCCGACCA TGGCTGGCGG GCGGCGTGCC GGTCGAATCC GGCACTAGCC 1080

AAAGGTCTTT CGACGCACGA AGGGGCGTTA CTGTCCGAAC GGGTGGCCAC CGACCTGGGG 1140

GTGCCGTTCA CCGAGCCCGC CAGCGTGCTG GCCTGACTCT CGGCCGCTCG TTACGCCGAG 1200

CACACGTCGG GAGTAAGGGA AGCGATGATG TCGGCC 1236

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

ATGCGCGTCG GTATTCCG 18

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

GCGCGTCGGT ATTCCGACCG 20

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

GAGACCAAAA ACAACGAA 18

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

GAATTCCCAT CAGCAATCTT GCAGA 25

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

GCCCCGATGA GCGAAGTC 18

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

GGGGCCGTCC TGGTGCC 17

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

GACGTCGACC TACGCGCTGA C 21

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

CTCGGTGAAC GGCACCCC 18

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

GGCCAGCACG CTGGCGGG 18

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

CACCCGTTCG GACAGTAA 18

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

CGCGGCCGAC ATCATCGC 18

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

GGCCGACATC ATCGCTTCCC 20

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

CGAGACTAAT TTGGGTGCCT TGGC 24

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 base pairs

(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

ATTTGGGTGC CTTGGC 16

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

GGCGGCGAGT CGACCGGC 18

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

AACGAATTCC AATTCCGGGT G 21

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

Asn Glu Phe Gln Phe Arg Val
1 5

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

AACGAATTCC GGGTG 15

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

Asn Glu Phe Arg Val
1 5